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(57) Abstract

A retroviral vector particle having a modified envelope polypeptide wherein a portion of the receptor binding region of the envelop is replaced with a targeting polypeptide which binds to a ligand or a receptor on the targeted cells. The targeting polypeptide may be single chain antibody. Such retroviral vector particles are useful in delivering genes encoding therapeutic agents to desired target cells c tissues *in vivo*.

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MODIFIED VIRAL ENVELOPE POLYPEPTIDE

This application is a continuation-in-part of application Serial No. 08/409,648, filed March 24, 1995.

This invention relates to a polypeptide which is a modified viral envelope. This invention further relates to "targeted" retroviral vector particles. More particularly, this invention relates to retroviral vector particles having a modified, or chimeric, envelope polypeptide wherein a portion of the envelope polypeptide is replaced with a polypeptide which binds to a ligand or receptor of a targeted cell. The term "polypeptide" as used herein means a polymer of amino acids and does not refer to any particular length of the polymer. Such term also includes post-translationally modified polypeptides or proteins (e.g., glycosylated, acetylated, phosphorylated, etc.).

BACKGROUND OF THE INVENTION

Retroviral vector particles are useful agents for introducing polynucleotides into cells, such as eukaryotic cells. The term "introducing" as used herein encompasses a variety of methods of transferring polynucleotides into a cell, such methods including transformation, transduction, transfection, and transinfection.

Retroviruses typically have three common open reading frames, gag, pol, and env, which encode the structural proteins, encode enzymes including reverse transcriptase, and

encode envelope proteins, respectively. Typically, retroviral vector particles are produced by packaging cell lines that provide the necessary gag, pol, and env gene products *in trans*. (Miller, et al., Human Gene Therapy, Vol. 1, pgs. 5-14 (1990)). This approach results in the production of retroviral vector particles which transduce mammalian cells, but are incapable of further replication after they have integrated into the genome of the cell.

Thus, retroviral vector particles have been used for introducing polynucleotides into cells for gene therapy purposes. In one approach, cells are obtained from a patient, and retroviral vector particles are used to introduce a desired polynucleotide into the cells, and such modified cells are returned to the patient with the engineered cells for a therapeutic purpose. In another approach, retroviral vector particles may be administered to the patient *in vivo*, whereby the retroviral vector particles transduce cells of the patient *in vivo*.

In many gene therapy protocols, it would be desirable to target retroviral vector particle infection to a specific population of cells either *in vivo* or *in vitro*. In such circumstances, the broad host range of typical retroviruses present a significant problem. A key determinant of viral host range is the "envelope" or "env" protein (encoded by the *env* gene) which is involved in binding to receptors on the surface of susceptible cells. Where it is possible to purify the desired target cells, either before or after transduction, such purification necessitates undesirable manipulations of the cells and may be problematic in situations in which the preferred target cells either are difficult to purify or are present at low or variable frequencies in mixed cell populations. Thus, it would be advantageous to have retroviral vector particles which could infect particular types of mammalian cells.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention now will be described with respect to the drawings, wherein:

Figure 1 is a schematic of the polypeptide (SEQ ID NO:1);

Figure 2 is a schematic of the polypeptide (SEQ ID NO:2); and

Figure 3 is a graph of relative percentage of melanin produced by B16-F1 cells stimulated with media from COS7 cells transfected with one of plasmids pcDNA-EF, p3-1, p3-2, or p6-3.

DETAILED DESCRIPTION OF THE INVENTION

Applicants have found that retroviral vector particles can be targeted to desired cells by providing the retroviral vector particle with a chimeric polypeptide which is derived from a viral envelope. More particularly, the chimeric polypeptide is produced by deleting specific portions of the polypeptide which comprises the receptor binding portion of a viral envelope hereinafter described, and replacing the deleted portions, hereinafter described, with a targeting polypeptide which binds to a receptor or ligand on the targeted cells.

More particularly, in accordance with an aspect of the present invention, there is provided a retroviral vector particle having a modified envelope polypeptide for targeting the retroviral vector particle to cells. Prior to modification, the envelope includes a receptor binding region which is a polypeptide selected from the group consisting of (a) a polypeptide having the sequence (SEQ ID NO:1), which is the receptor-binding region of an ecotropic retroviral envelope; (b) a polypeptide having the sequence (SEQ ID NO:2), which is the receptor-binding region of an amphotropic retroviral envelope; (c) a polypeptide having the sequence (SEQ ID NO:3), which is the receptor binding region of 10A1 murine leukemia virus envelope; (d) a polypeptide having the

sequence (SEQ ID NO:4), which is the receptor-binding region of murine leukemia virus NZB-9-1 xenotropic envelope; and (e) a polypeptide having the sequence (SEQ ID NO:5), which is the receptor-binding region of murine leukemia virus polytropic MX27 provirus envelope. When, prior to modification, the ecotropic envelope includes the polypeptide having (SEQ ID NO:1), in the modified envelope at least a portion of (i) amino acids 70 to 92 of (SEQ ID NO:1); or (ii) amino acids 44 to 114 of (SEQ ID NO:1); or (iii) amino acids 44 to 131 of (SEQ ID NO:1); or (iv) amino acids 17 to 182 of (SEQ ID NO:1) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the amphotropic envelope includes the polypeptide having (SEQ ID NO:2), in the modified envelope at least a portion of (i) amino acids 47 to 75 of (SEQ ID NO:2); or (ii) amino acids 47 to 93 of (SEQ ID NO:2); or (iii) amino acids 47 to 163 of (SEQ ID NO:2) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

When, prior to modification, the envelope includes the polypeptide having (SEQ ID NO:3), in the modified envelope at least a portion of (i) amino acids 47 to 75 of (SEQ ID NO:3); or (ii) amino acids 47 to 93 of (SEQ ID NO:3); or (iii) amino acids 47 to 163 of (SEQ ID NO:3) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the xenotropic envelope includes the polypeptide having (SEQ ID NO:4), in the modified envelope at least a portion of (i) amino acids 47 to 74 of (SEQ ID NO:4); or (ii) amino acids 47 to 92 of (SEQ ID NO:4); or (iii) amino acids 47 to 154 of (SEQ ID NO:4) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the polytropic envelope includes the polypeptide having (SEQ ID NO:5), in the modified envelope at least a portion of (i) amino acids 47 to 70 of (SEQ ID NO:5);

or (ii) amino acids 47 to 88 of (SEQ ID NO:5); or (iii) amino acids 47 to 151 of (SEQ ID NO:5) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

In one embodiment, the envelope, prior to modification, includes the polypeptide having (SEQ ID NO:1), and at least a portion, of amino acids 70 to 92 of (SEQ ID NO:1) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. In one embodiment, all of amino acids 70 to 92 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In another embodiment, at least a portion of amino acids 74 to 91 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In one embodiment, all of amino acids 74 to 91 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In another embodiment, amino acids 80 to 88 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In yet another embodiment, amino acids 82 to 84 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In a further embodiment, amino acids 74 to 80 (of SEQ ID NO:1) are replaced with a targeting polypeptide. In another embodiment, at least a portion, and preferably all, of amino acids 44 to 114 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In yet another embodiment, at least a portion, and preferably all, of amino acids 44 to 131 of (SEQ ID NO:1) are replaced with a targeting polypeptide. In a further embodiment, at least a portion, and preferably all, of amino acids 17 to 182 of (SEQ ID NO:1) are replaced with a targeting polypeptide.

In another embodiment, the envelope, prior to modification, includes the polypeptide having (SEQ ID NO:2), and at least a portion, and preferably all, of amino acids 47 to 75 of (SEQ ID NO:2) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. In another embodiment, at least a portion, and preferably all, of amino acids 47 to 93 of (SEQ ID NO:2)

are replaced. In yet another embodiment, at least a portion, and preferably all, of amino acid residues 47 to 163 of (SEQ ID NO:2) are replaced.

In another embodiment, the envelope, prior to modification, includes the polypeptide having (SEQ ID NO:3), and at least a portion, and preferably all of amino acids 47 to 75 of (SEQ ID NO:3) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. In another embodiment, at least a portion, and preferably all, of amino acids 47 to 93 of (SEQ ID NO:3) is replaced. In yet another embodiment, at least a portion, and preferably all, of amino acids 47 to 163 of (SEQ ID NO:3) is replaced.

In a further embodiment, the envelope, prior to modification, includes the polypeptide having (SEQ ID NO:4), and at least a portion, and preferably all, of amino acids 47 to 74 of (SEQ ID NO:4) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. In another embodiment, at least a portion, and preferably all, of amino acids 47 to 92 of (SEQ ID NO:4) is replaced. In yet another embodiment, at least a portion, and preferably all, of amino acids 47 to 154 of (SEQ ID NO:4) is replaced.

In another embodiment, the envelope, prior to modification, includes the polypeptide having (SEQ ID NO:5), and at least a portion, and preferably all, of amino acids 47 to 70 of (SEQ ID NO:5) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells. In another embodiment, at least a portion, and preferably all, of amino acids 47 to 88 of (SEQ ID NO:5) is replaced. In yet another embodiment, at least a portion, and preferably all, of amino acids 47 to 151 of (SEQ ID NO:5) is replaced.

In general, the polypeptides (SEQ ID NO:1) through (SEQ ID NO:5) are portions of a protein known as gp 70, which is

included in the envelope of murine leukemia viruses. In general, gp 70 protein includes the following regions: (i) the secretory signal or "leader" sequence; (ii) the receptor binding region; (iii) the hinge region; and (iv) the body portion. The polypeptides (SEQ ID NO:1) and (SEQ ID NO:2) are receptor binding regions of ecotropic envelope of Moloney Murine Leukemia Virus; and amphotropic retroviral envelope of 4070A retrovirus, respectively. (SEQ ID NO:3) is the receptor binding region of the 10A1 murine leukemia virus envelope, and also is described in Ott, et al., J. Virol., Vol. 64, pgs. 757-766 (1990). The nucleic acid sequence of such envelope is registered as GenBank Accession No. M33470. (SEQ ID NO:4) is the receptor binding region of murine leukemia virus NZB-9-1 xenotropic envelope, the nucleic acid sequence of which is registered as GenBank Accession No. KO2730 and described in O'Neill, et al., J. Virol., Vol. 53, pgs. 100-106 (1985). (SEQ ID NO:5) is the receptor binding region of mouse murine leukemia virus polytropic MX27 provirus, the nucleic acid sequence of which is registered as GenBank Accession No. M17326, and is described in Stoye, et al., J. Virol., Vol. 61, pgs. 2659-2669 (1987). Applicants have found that retroviruses can be made "targetable" to a specific type of cell if a portion of the receptor binding region is modified such that the receptor binding region includes a polypeptide which binds to a ligand or receptor of a target cell. Although the retroviral vector particles of the present invention include a modified receptor binding region of the envelope protein, such retroviral particles also may have additional modifications in other regions of the envelope protein, such as, for example, the secretory signal or "leader" sequence, the hinge region, or the body portion. Such modifications may include deletions and/or substitutions.

Targeting polypeptides which may be employed include, but are not limited to, antibodies and fragments thereof, including single-chain antibodies, monoclonal antibodies, and

polyclonal antibodies. Such antibodies include, but are not limited to, antibodies and fragments or portions thereof which bind to erb-B2, such as, for example, e23 antibody; antibodies which bind to receptors such as, for example, the CD4 receptor on T-cells; antibodies which bind to the transferrin receptor; antibodies directed against human leukocyte antigen (HLA): antibodies to carcinoembryonic antigen; antibodies to placental alkaline phosphatase found on testicular and ovarian cancer cells; antibodies to polymorphic epithelial mucin found on ovarian cancer cells; antibodies to β-human chorionic gonadotropin; antibodies to CD20 antigen of B-lymphoma cells; antibodies to alphafetoprotein; antibodies to prostate specific antigen; OKT-3 antibody, which binds to CD3 T-lymphocyte surface antigen; antibodies which bind to B-lymphocyte surface antigen; antibodies which bind to EGFR (c-erb-B1 or c-erb-B2) found on glioma cells, B-cell lymphoma cells; and breast cancer cells; anti-tac monoclonal antibody, which binds to the Interleukin-2 receptor; anti-transferrin monoclonal antibodies; monoclonal antibodies to gp 95/gp 97 found on melanoma cells; monoclonal antibodies to p-glycoproteins; monoclonal antibodies to cluster-1 antigen (N-CAM), cluster-w4, cluster-5A, or cluster-6 (LeY), all found on small cell lung carcinomas; monoclonal antibodies to placental alkaline phosphatase; monoclonal antibodies to CA-125 found on lung and ovarian carcinoma cells, monoclonal antibodies to epithelial specific antigen (ESA) found on lung and ovarian carcinoma cells; monoclonal antibodies to CD19, CD22, and CD37 found on B-cell lymphoma cells; monoclonal antibodies to the 250 kDa proteoglycan found on melanoma cells; monoclonal antibodies to p55 protein found on breast cancer cells; monoclonal antibodies to the TCR-IgH fusion protein found on childhood T-cell leukemia cells; antibodies to T-cell antigen receptors; antibodies to tumor specific antigen on B-cell lymphomas; antibodies to organ cell surface markers; anti-HIV

antibodies, such as anti-HIV gp 120-specific immunoglobulin, and anti-erythrocyte antibodies.

Other targeting peptides which may be employed include cytokines. Such cytokines include, but are not limited to, interleukins, including Interleukin-1α, Interleukin 1β, and Interleukins 2 through 14; growth factors such as epithelial growth factor (EGF), TGF-α, TGF-β, fibroblast growth factor (FGF), keratinocyte growth factor (KGF), PDGF-A, PDGF-B, PD-ECGF, IGF-I, IGF-II, and nerve growth factor (NGF), which binds to the NGF receptor of neural cells; colony stimulating factors such as GM-CSF, G-CSF, and M-CSF, leukemic inhibitory factor (LIF); interferons such as interferon-α, interferon-β, and interferon-γ; inhibin A; inhibin B; chemotactic factors; α-type intercrine cytokines; and β-type intercrine cytokines.

Still other targeting polypeptides which may be employed include, but are not limited to, melanotropin stimulating hormones, which bind to the MSH receptor on melanoma cells, such as, for example, alpha-melanotropin stimulating hormone or alpha-MSH; erythropoietin, which binds to the erythropoietin receptor; adhesins; selectins; CD34, which binds to the CD34 receptor of hematopoietic stem cells; CD33, which binds to premyeloblastic leukemia cells; stem cell factor; integrins; asialoglycoproteins, including asialoorosomucoid, asialofetuin, and alpha-1 acid glycoprotein, which binds to the asialoglycoprotein receptor of liver cells; insulin; glucagon; gastrin polypeptides, which bind to receptors on hematopoietic stem cells; C-kit ligand; tumor necrosis factors (or TNF's) such as, for example, TNF-alpha and TNF-beta; ApoB, which binds to the LDL receptor of liver cells; alpha-2-macroglobulin, which binds to the LRP receptor of liver cells; mannose-containing peptides, which bind to the mannose receptor of macrophages; sialyl-Lewis-X antigen-containing peptides, which bind to the ELAM-1 receptor of activated endothelial cells; CD40 ligand, which binds to the CD40 receptor of B-lymphocytes; ICAM-1,

which binds to the LFA-1 (CD11b/CD18) receptor of lymphocytes, or to the Mac-1 (CD11a/CD18) receptor of macrophages; M-CSF, which binds to the c-fms receptor of spleen and bone marrow macrophages; VLA-4, which binds to the VCAM-1 receptor of activated endothelial cells; LFA-1, which binds to the ICAM-1 receptor of activated endothelial cells; HIV gp120 and Class II MHC antigen, which bind to the CD4 receptor of T-helper cells; and the LDL receptor binding region of the apolipoprotein E (ApoE) molecule. It is to be understood, however, that the scope of the present invention is not to be limited to any specific targeting polypeptide.

In one embodiment, the targeting polypeptide is a single chain antibody.

Thus, in accordance with another aspect of the present invention, there is provided a modified polynucleotide encoding a modified retroviral envelope polypeptide for targeting a retroviral vector to cells. Prior to modification, the envelope includes a polypeptide selected from the group consisting of (a) a polypeptide having the sequence (SEQ ID NO:1); (b) a polypeptide having the sequence (SEQ ID NO:2); (c) a polypeptide having the sequence (SEQ ID NO:3); (d) a polypeptide having the sequence (SEQ ID NO:4); and (e) a polypeptide having the sequence (SEQ ID NO:5). When, prior to modification, the ecotropic envelope includes (SEQ ID NO:1), in the modified polynucleotide, at least a portion of (i) the polynucleotide encoding amino acids 70 to 92 of (SEQ ID NO:1); or (ii) the polyncleotide encoding amino acids 47 to 93 of (SEQ ID NO:3); or (iii) the polynucleotide encoding amino acids 44 to 114 of (SEQ ID NO:1); or (iii) the polynucleotide encoding amino acids 44 to 131 of (SEQ ID NO:1); or (iv) the polynucleotide encoding amino acids 17 to 182 of (SEQ ID NO:1) is removed and replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the amphotropic envelope includes (SEQ ID

NO:2), in the modified polynucleotide at least a portion of (i) the polynucleotide encoding amino acids 47 to 75 of (SEQ ID NO:2); or (ii) the polynucleotide encoding amino acids 47 to 93 of (SEQ ID NO:2); or (iii) the polynucleotide encoding amino acids 47 to 163 of (SEQ ID NO:2) is replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the envelope includes the polypeptide having (SEQ ID NO:3), in the modified polynucleotide at least a portion of (i) the polynucleotide encoding amino acids 47 to 75 of (SEQ ID NO:3); or (ii) the polynucleotide encoding amino acids 47 to 93 of (SEQ ID NO:3); or (iii) the polynucleotide encoding amino acids 47 to 163 of (SEQ ID NO:3) is removed and replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the xenotropic envelope includes the polypeptide having (SEQ ID NO:4), in the modified polynucleotide at least a portion of the polynucleotide encoding (i) amino acids 47 to 74 of (SEQ ID NO:4); or (ii) the polynucleotide encoding amino acids 47 to 92 of (SEQ ID NO:4); or (iii) the polynucleotide encoding amino acids 47 to 154 of (SEQ ID NO:4)is removed and replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells. When, prior to modification, the polytropic envelope includes the polypeptide having (SEQ ID NO:5) in the modified polynucleotide at least a portion of (i) the polynucleotide encoding amino acids 47 to 70 of (SEQ ID NO:5); or (ii) the polynucleotide encoding amino acids 47 to 88 of (SEQ ID NO:5); or (iii) the polynucleotide encoding amino acids 47 to 151 of (SEQ ID NO:5) is removed and replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells. The polypeptide (SEQ ID NO:1) is encoded by the polynucleotide having (SEQ ID NO:6) or a derivative or analogic thereof.

The polypeptide (SEQ ID NO:2) is encoded by the polynucleotide having (SEQ ID NO:7) or a derivative or analogue thereof. The polypeptide (SEQ ID NO:3) is encoded by the polynucleotide having (SEQ ID NO:8) or a derivative or analogue thereof. The polypeptide (SEQ ID NO:4) is encoded by the polynucleotide having (SEQ ID NO:9) or a derivative or analogue thereof. The polypeptide (SEQ ID NO:5) is encoded by the polynucleotide having (SEQ ID NO:10) or a derivative or analogue thereof. The term "derivative or analogue thereof" as used herein means that the polynucleotides encoding one of the polypeptides (SEQ ID NO:1) through (SEQ ID NO:5) may have a sequence different from one of polynucleotides (SEQ ID NO:6) through (SEQ ID NO:10), yet encode the same polypeptide. Such differences in the polynucleotide sequences may, for example, be due to the degeneration of the genetic code. Such a polynucleotide may be constructed by genetic engineering techniques known to those skilled in the art.

For example, a first expression plasmid may be constructed which includes a polynucleotide encoding the unmodified envelope. The plasmid then is engineered such that a polynucleotide encoding an amino acid sequence as hereinabove described may be removed, and to provide appropriate restriction enzyme sites for removal of the polynucleotide sequence encoding an amino acid sequence as hereinabove described, and replacement of such polynucleotide sequence with a polynucleotide sequence encoding a targeting polypeptide. The polynucleotide encoding the targeting polypeptide may be contained in a second expression plasmid or may exist as a naked polynucleotide sequence. The polynucleotide encoding the targeting polypeptide or the plasmid containing such polynucleotide is cut at appropriate restriction enzyme sites and cloned into the first expression plasmid which also has been cut at appropriate restriction enzyme sites. The resulting expression plasmid thus includes

a polynucleotide encoding the modified envelope protein. Such polynucleotide then may be cloned out of the expression plasmid, and into a retroviral plasmid vector. The resulting retroviral plasmid vector, which includes the polynucleotide encoding the modified envelope protein, and which also may include a polynucleotide encoding a heterologous protein or peptide, is transfected into an appropriate packaging cell line to form a producer cell line for generating retroviral vector particles including the modified envelope protein. Alternatively, a naked polynucleotide sequence encoding the modified envelope protein is transfected into a "pre-packaging" cell line including nucleic acid sequences encoding the gag and pol proteins, thereby forming a packaging cell line, or is transfected into a packaging cell line including nucleic acid sequences encoding the gag, pol, and wild-type (i.e., unmodified) env proteins, thereby forming a packaging cell line including nucleic acid sequences encoding wild-type env protein and the modified envelope protein. Such packaging cells then may be transfected with a retroviral plasmid vector, which may include a nucleic acid sequence encoding a heterologous protein or peptide, thereby forming a producer cell line for generating retroviral vector particles including the modified envelope protein. Such a polynucleotide thus may be contained in the above-mentioned retroviral vector particle, or in a producer cell for generating the above-mentioned retroviral vector particle.

The term "polynucleotide" as used herein means a polymeric form of nucleotide of any length, and includes ribonucleotides and deoxyribonucleotides. Such term also includes single- and double-stranded DNA, as well as single- and double-stranded RNA. The term also includes modified polynucleotides such as methylated or capped polynucleotides.

In a preferred embodiment, the retroviral vector particle having a modified envelope in accordance with the

invention includes a polynucleotide encoding a heterologous polypeptide which is to be expressed in a targeted cell. The heterologous polypeptide may, in one embodiment, be a therapeutic agent. The term "therapeutic" is used in a generic sense and includes treating agents, prophylactic agents, and replacement agents.

Polynucleotides encoding therapeutic agents which may be contained in the retroviral vector particle include, but are not limited to, polynucleotides encoding tumor necrosis factor (TNF) genes, such as TNF-α; genes encoding interferons such as Interferon-α, Interferon-β, and Interferon-γ; genes encoding interleukins such as IL-1, IL-1β, and Interleukins 2 through 14; genes encoding GM-CSF; genes encoding adenosine deaminase, or ADA; genes which encode cellular growth factors, such as lymphokines, which are growth factors for lymphocytes; genes encoding epidermal growth factor (EGF), and keratinocyte growth factor (KGF); genes encoding soluble CD4; Factor VIII; Factor IX; cytochrome b; glucocerebrosidase; T-cell receptors; the LDL receptor, ApoE, ApoC, ApoAI and other genes involved in cholesterol transport and metabolism; the alpha-1 antitrypsin (α1AT) gene; the insulin gene; the hypoxanthine phosphoribosyl transferase gene; negative selective markers or "suicide" genes, such as viral thymidine kinase genes, such as the Herpes Simplex Virus thymidine kinase gene, the cytomegalovirus virus thymidine kinase gene, and the varicella-zoster virus thymidine kinase gene; Fc receptors for antigen-binding domains of antibodies, antisense sequences which inhibit viral replication, such as antisense sequences which inhibit replication of hepatitis B or hepatitis non-A non-B virus; antisense *c-myb* oligonucleotides; and antioxidants such as, but not limited to, manganese superoxide dismutase (Mn-SOD), catalase, copper-zinc-superoxide dismutase (CuZn-SOD), extracellular superoxide dismutase (EC-SOD), and glutathione reductase; tissue plasminogen activator (tPA); urinary

plasminogen activator (urokinase); hirudin; the phenylalanine hydroxylase gene; nitric oxide synthesase; vasoactive peptides; angiogenic peptides; the dopamine gene; the dystrophin gene; the β-globin gene; the α-globin gene; the HbA gene; protconcogenes such as the *ras*, *src*, and *bcl* genes; tumor-suppressor genes such as p53 and Rb; the LDL receptor; the heregulin-α protein gene, for treating breast, ovarian, gastric and endometrial cancers; monoclonal antibodies specific to epitopes contained within the β-chain of a T-cell antigen receptor; the multidrug resistance (MDR) gene; polynucleotides encoding ribozymes; antisense polynucleotides; genes encoding secretory peptides which act as competitive inhibitors of angiotensin converting enzyme, of vascular smooth muscle calcium channels, or of adrenergic receptors, and polynucleotides encoding enzymes which break down amyloid plaques within the central nervous system. It is to be understood, however, that the scope of the present invention is not to be limited to any particular therapeutic agent.

The polynucleotide encoding the therapeutic agent is under the control of a suitable promoter. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; the cytomegalovirus (CMV) promoter; the Rous Sarcoma Virus (RSV) promoter; the histone promoter; the polIII promoter, the β-actin promoter; inducible promoters, such as the MMTV promoter, the metallothionein promoter; heat shock promoters; adenovirus promoters; the albumin promoter; the ApoAI promoter; B19 parvovirus promoters; human globin promoters; viral thymidine kinase promoters, such as the Herpes Simplex thymidine kinase promoter; retroviral LTRs; human growth hormone promoters, and the MxIFN inducible promoter. The promoter also may be the native promoter which controls the polynucleotide encoding the therapeutic agent. It is to be understood,

however, that the scope of the present invention is not to be limited to specific foreign genes or promoters.

The polynucleotides encoding the modified envelope polypeptide and the therapeutic agent may be placed into an appropriate retroviral plasmid vector by genetic engineering techniques known to those skilled in the art.

In one embodiment, the retroviral plasmid vector may be derived from Moloney Murine Leukemia Virus and is of the LN series of vectors, such as those hereinabove mentioned, and described further in Bender, *et al.*, J. Virol., Vol. 61, pgs. 1639-1649 (1987) and Miller, *et al.*, Biotechniques, Vol. 7, pgs 980-990 (1989). Such vectors have a portion of the packaging signal derived from a mouse sarcoma virus, and a mutated gag initiation codon. The term "mutated" as used herein means that the gag initiation codon has been deleted or altered such that the gag protein or fragments or truncations thereof, are not expressed.

In another embodiment, the retroviral plasmid vector may include at least four cloning, or restriction enzyme recognition sites, wherein at least two of the sites have an average frequency of appearance in eukaryotic genes of less than once in 10,000 base pairs; i.e., the restriction product has an average DNA size of at least 10,000 base pairs. Preferred cloning sites are selected from the group consisting of NotI, SnaBI, SalI, and XhoI. In a preferred embodiment, the retroviral plasmid vector includes each of these cloning sites. Such vectors are further described in U.S. Patent Application Serial No. 08/340,805, filed November 17, 1994, and in PCT Application No. W091/10728, published July 25, 1991, and incorporated herein by reference in their entireties.

When a retroviral plasmid vector including such cloning sites is employed, there may also be provided a shuttle cloning vector which includes at least two cloning sites which are compatible with at least two cloning sites selected

from the group consisting of NotI, SnaBI, SalI, and XhoI located on the retroviral plasmid vector. The shuttle cloning vector also includes at least one desired polynucleotide encoding a therapeutic agent which is capable of being transferred from the shuttle cloning vector to the retroviral plasmid vector.

The shuttle cloning vector may be constructed from a basic "backbone" vector or fragment to which are ligated one or more linkers which include cloning or restriction enzyme recognition sites. Included in the cloning sites are the compatible, or complementary cloning sites hereinabove described. Genes and/or promoters having ends corresponding to the restriction sites of the shuttle vector may be ligated into the shuttle vector through techniques known in the art.

The shuttle cloning vector can be employed to amplify DNA sequences in prokaryotic systems. The shuttle cloning vector may be prepared from plasmids generally used in prokaryotic systems and in particular in bacteria. Thus, for example, the shuttle cloning vector may be derived from plasmids such as pBR322; pUC 18; etc.

The retroviral plasmid vector includes one or more promoters. Suitable promoters which may be employed include, but are not limited to, the retroviral LTR; the SV40 promoter; and the human cytomegalovirus (CMV) promoter described in Miller, *et al.*, Biotechniques, Vol. 7, No. 9, 980-990 (1989), or any other promoter (e.g., cellular promoters such as eukaryotic cellular promoters including, but not limited to, the histone, pol III, and β-actin promoters). Other viral promoters which may be employed include, but are not limited to, adenovirus promoters, TK promoters, and B19 parvovirus promoters. The selection of a suitable promoter will be apparent to those skilled in the art from the teachings contained herein.

In one embodiment, the retroviral plasmid vector, which includes a polynucleotide encoding the modified envelope and

a polynucleotide encoding a therapeutic agent, is employed to transduce a packaging cell line to form a producer cell line, which will generate infectious retroviral vector particles. In one embodiment, the packaging cell line is a "pre-packaging" cell line which includes polynucleotides encoding the gag and pol retroviral proteins, but not the envelope, or env, protein. Examples of such "pre-packaging" cell lines include, but are not limited to, GP8 cells, GPL cells, and GPNZ cells as described in Morgan, *et al.*, J. Virol., Vol. 67, No. 8, pgs. 4712-4721 (August 1993). Such cell lines, upon transduction with the retroviral plasmid vector, generates infectious retroviral particles including the modified, or chimeric, envelope and a polynucleotide encoding the therapeutic agent.

In another embodiment, a retroviral plasmid vector which includes a polynucleotide encoding a modified polynucleotide encoding a modified envelope polypeptide in accordance with the invention and a polynucleotide encoding a therapeutic agent is used to transduce a packaging cell line including nucleic acid sequences encoding the gag, pol, and wild-type (i.e., unmodified) env retroviral proteins. Examples of such packaging cell lines include, but are not limited to, the PE501, PA317 (ATCC No. CRL 9078), ψ-2, ψ-AM, PA12, T19-14X, VT-19-17-H2, ψCRE, ψCRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990), which is incorporated herein by reference in its entirety. The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, and use of liposomes, such as hereinabove described, and $CaPO_4$ precipitation. Such producer cells generate infectious retroviral vector particles which include the modified envelope, the wild-type retroviral envelope, a polynucleotide encoding the modified, or chimeric, envelope, and a polynucleotide encoding a therapeutic agent.

In another embodiment, there is provided a packaging cell which includes a nucleic acid sequence encoding a modified chimeric envelope in accordance with the invention, and which may further include nucleic acid sequences encoding the gag and pol proteins. A producer cell for generating viral particles which includes a modified envelope in accordance with the invention is produced by introducing into such packaging cell either a retroviral vector particle or a retroviral plasmid vector, in each case including a polynucleotide encoding a therapeutic agent. The producer cell line thus generates infectious retroviral particles including the modified chimeric envelope and the polynucleotide encoding the therapeutic agent.

The retroviral vector particles, which include the modified envelope, and a polynucleotide encoding a therapeutic agent, may be administered to a host in an amount effective to produce a therapeutic effect in the host. The host may be a mammalian host, which may be a human or non-human primate host. The retroviral vector particles, upon administration to the host, travel to and transduce the desired target cells, whereby the transduced target cells express the therapeutic agent *in vivo*. The exact dosage of retroviral vector particles which may be administered is dependent upon a variety of factors, including the age, sex, and weight of the patient, the target cells which are to be transduced, the therapeutic agent which is to be administered, and the severity of the disorder to be treated.

The retroviral vector particles may be administered sytemically, such as, for example, by intravenous, or intraperitoneal administration, as well as by intranasal, intratracheal, endotracheal, intraarterial, intravesicular, or intracolonic administration.

Cells which may be transduced with the retroviral vector particles of the present invention include, but are not limited to, primary cells, such as primary nucleated blood

cells, primary tumor cells, endothelial cells, epithelial cells, keratinocytes, stem cells, hepatocytes, connective tissue cells, fibroblasts, mesenchymal cells, mesothelial cells, and parenchymal cells; stem cells, such as hematopoietic stem cells; T-lymphocytes; B-lymphocytes; neutrophils; macrophages; platelets; erythrocytes; nerve cells; brain cells; muscle cells; lung cells, pancreatic cells; and malignant and non-malignant tumor cells. The selection of the particular cells which are to be transduced is dependent upon the disease or disorder to be treated as well as the targeting polypeptide contained in the modified envelope. It is to be understood that the scope of the present invention is not to be limited to the transduction of any specific target cells.

Diseases or disorders which may be treated with the retroviral vector particles of the present invention include, but are not limited to, severe combined immune deficiency caused by adenosine deaminase deficiency; sickle cell anemia; thalassemia; hemophilia; diabetes; emphysema caused by α-1-antitrypsin deficiency; Alzheimer's disease; AIDS; chronic granulomatosis; Gaucher's disease; Lesch-Nyhan syndrome; muscular dystrophy, including Duchenne muscular dystrophy; Parkinson's disease; cystic fibrosis; phenylketonuria; hypercholesterolemia; and other illnesses such as growth disorders and heart diseases, such as, for example, those caused by alterations in the way cholesterol is metabolized and defects in the immune system.

The retroviral vector particles also may be employed in the treatment of tumors, including malignant and non-malignant tumors. For example, a retroviral vector particle including a modified envelope protein, including a targeting polypeptide which binds to a tumor cell, and a polynucleotide encoding a negative selective marker or "suicide" gene, such as, for example, the Herpes Simplex thymidine kinase (TK) gene, may be administered to a patient, whereby the

retroviral vector particles transduce the tumor cells. After the tumor cells are transduced with the retroviral vector particles, an interaction agent, such as gancyclovir or acyclovir, is administered to the patient, whereby the transduced tumor cells are killed.

It is to be understood that the present invention is not to be limited to the treatment of any particular disease or disorder.

The retroviral vector particles, which include the modified envelope protein and a polynucleotide encoding a therapeutic agent, may be administered to an animal *in vivo* as part of an animal model for the study of the effectiveness of a gene therapy treatment. The retroviral vector particles may be administered in varying doses to different animals of the same species, whereby the retroviral vector particles will transduce the desired target cells in the animal. The animals then are evaluated for the expression of the desired therapeutic agent *in vivo* in the animal. From the data obtained from such evaluations, one may determine the amount of retroviral vector particles to be administered to a human patient.

The retroviral vector particles of the present invention also may be employed in the *in vitro* transduction of desired target cells, which are contained in a cell culture containing a mixture of cells. Upon transduction of the target cells *in vitro*, the target cells produce the therapeutic agent or protein *in vitro*. The therapeutic agent or protein then may be obtained from the cell culture by means known to those skilled in the art.

The retroviral vector particles also may be employed for the transduction of cells *in vitro* in order to study the mechanism of the genetic engineering of cells *in vitro*.

In addition, the modified envelope polypeptides of the present invention may be employed to form proteoliposomes; i.e., the modified envelope polypeptide forms a portion of

the liposome wall. Such proteoliposomes may be employed for gene transfer or for drug delivery to desired target cells.

The invention now will be described with respect to the following examples; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Construction of scFV-env chimeras

In this example, all polymerase chain reactions (PCR) were done using a Gene Amp kit in a model 9600 thermal cycler (Perkin Elmer). In the following example, the following oligonucleotides were employed in plasmid constructions.

Name	Sequence (5'-->3')	Description
AD191	CCGGAATTCAGATCTTATATGGG	Mutagenic oligo to create Bgl II site immediately 3' to EcoR I site separating CMV promoter and env gene
AD196	CCAGCCAATTGCTCCGTGG	Mutagenic oligo to create Mun I site by mutating 3rd position of codon of amino acid Asn_{333} of env protein
AD249	CCGGGGGCCCAGCCGGCCGCGGCCGCC	Top strand of Xma I-Sfi I-Not I-Xma linker
AD250	CCGGGGGCGGCCGCGGCCGGCTGGGCCC	Bottom strand complement of AD249
AD275	TTGGCCCAGCCGGCCATGGACCTGCAGCT GACCC	Oligo to add Sfi I site to 5'-end of e23 by PCR
AD276	TTGCGGCCGCGGAGACGGTGACCGTGGT	Oligo to add Not I site to 3'-end of e23 by PCR
AD15	ATGTAAGCCCTGGATCTTGTCCGG	oligo for splicing overlap extention (SOE) PCR
AD16	ATGGGCAACTTCTGGCAACCACCC	same as above
AD109	GGTCTTTAATGTAACCTGGAGAGTCACC	same as above
AD111	GGCCACAGGCAACTTTAGAGCATCC	same as above
AD287	CCTCATCAAGTCTATCAGATCACCTGGGAGG	same as above
AD416	CGCTCTCAAAACCCCCTC	same as above
AD422	GTCAAAAGTACGCGTCCGCTGTCTCC	mutagenic oligo to create MluI site in CAE
AD423	TTAATTCGAGCTCGCCCCCGGAGTTTCGGG GGGATCC	mutagenic oligo to remove EcoRI site in CAE (2105bp)

AD604	GGACCTGGTGGCCAGATCTTACC	mutagenic oligo to create BglII in Cee+
AD605	GCAACACTGCCTGGAACAGGCTCAAGC	mutagenic oligo to create EcoNI site in Cee+
AD725	GGTTGTCTCACAGCCCCATTTGCCACAGT AGAAGG	oligo for splicing overlap overlap extention PCR
AD868	GATCTTACCCCAGGGGCCCAGCCGGCCGGA	top strand to add SfiI site in Cee+ to have E/S1
AD869	GATCTCCGGCCGGCTGGGCGCCTGGGGTAA	bottom strand complement of AD868
AD870	GGAGGGGCCCAGCCGGCCTGTGATCTGGTC GGAGAGGAGTGG	oligo for SOE PCR to create SfiI in A/S1
AD871	GGCCGGCTGGGCCCCTCCTAGATCAAAATA TAATTTTGGG	same as AD871
AD879	GGAGGGGCCCAGCCGGCCCCCCTTGTTGCT CAGGGGGC	oligo for SOE PCR to create SfiI in E/S2
AD880	GGCCGGCTGGGCCCCTCCCCCCGGGGGAGA AGAAAAAGG	same as AD879
AD881	ACCTCCCTCGCGGCCGCCACCCCTCGGTGC AACACTGC	oligo for SOE PCR to create NotI in E/S2N1
AD882	CCGAGGGGTGGCGGCCGCGAGGGAGGTTAA AGGTTCTTCG	same as AD881
AD883	TCAAATGAGGCGGCCGCCGGATTTTATGTTT GCCCC	oligo for SOE PCR to create NotI in E/S1N2
AD884	ATAAAATCCGGCGGCCGCCTCATTTGATTTA TGAGTTGTCTGG	same as AD883
AD885	TCCAAGTCATGTGCGGCCGCCGGGGGTCCAG ACTCCTTCTACTGTGC	oligo for SOE PCR to create NotI in E/S1N3
AD886	TGGACCCCCGGCGGCCGCACATGACTTGGAT TCTCGGGG	same as AD885
AD887	GTCGGGGTGTGCGGCCGCCGGGGGACCAGGA GAGGGC	oligo for SOE PCR to create NotI in A/S1N2
AD888	GGCGGCCGCACACCCCGACTTTACGGTATGC	same as AD887
AD889	CGCGTGCGGCCGCCAGTGGAGGAA	top strand to add NotI in A/S1N1
AD890	CGCGTTCCTCCACTGGCGGCCGCA	bottom strand complement of AD889
AD936	GTTACCCCGGCCGGCTGGGCCCCG	bottom strand complement of AD937
AD937	GTAACCGGGGCCCAGCCGGCCGGG	top strand to add SfiI to have E/S0
AD938	AATTCGCGGCCGCCAACCCTCTAGTCCTAG	top strand to add Not I to have A/S1N3

AD939 AATTCTAGGACTAGAGGGTTGGCGGCCGCG bottom strand to add NotI to have A/S1N3

Splicing overlap extension PCR reactions were performed using the method of Horton, *et al.*, Gene, Vol. 77, No. 1, pgs. 61-68) (1989). Plasmids were sequenced using the Sequenase 2.0 kit from USB.

Site-directed mutagenesis reactions were performed using single-stranded phagemid DNA according to the method of Morris, et al., Biochem. and Biophys. Res. Comm., Vol. 117, No. 1, pgs. 299-305 (1983). Template DNA for use in mutagenesis reactions was obtained by rescuing single-stranded DNA from Bluescript (Stratagene) derivatives by superinfection with a helper phage.

I. Construction of e23FV-env expression plasmids

Construction of Backbone Plasmids. Cee+ is a CMV-env expression vector constructed by digesting CEE (Morgan, *et al.*, 1993) with Hind III and Not I, filling in the Not I site with Klenow fragment, and ligating the CMV-env cassette into Bluescript II SK+ digested with Sma I and Hind III.

Cee+ΔNot was made by digesting Cee+ with Not I, removing the 5'-extensions by digesting with Mung Bean Nuclease and recircularizing the plasmid.

Single stranded phagemid DNA was made from *E. coli* JM109 transformed with Cee+ΔNot. 5μg of single-stranded DNA was mixed with 4pmol each of AD191 and AD196, and used as template for site directed mutagenesis to introduce Bgl II and Mun I sites. A plasmid, CEC, that had obtained the desired Bgl II and Mun I sites was identified by digestion with these enzymes, and confirmed by DNA sequencing. This plasmid served as the basis of all chimeric envelope expression vectors.

CEC was digested with Xma I and dephosphorylated. A phosphorylated double-stranded linker, made by heating and annealing AD249 and AD250, was ligated into this site. The resulting product was then digested with Not I, diluted and

recircularized by ligation. CECX, a plasmid containing a single copy of the polylinker oriented so that the Sfi I site was closer to the 5'-end of env, was identified by digestion with Sfi I and Not I and confirmed by DNA sequencing.

The same double stranded linker was added to Bluescript II SK(+) that had been digested with Xma I and dephosphorylated. Following ligation of the linker, the ligation mix was digested with Not I, ethanol precipitated, diluted and religated. pBSR(L) (for "linker") was identified by screening for the presence of an Sfi I site and the absence of an Spe I site, and confirmed by sequencing.

Addition of linker sequences to e23 scFv. DNA encoding e23 scFv single chain antibody which is an Erb-B2 single chain monoclonal antibody which is to be employed in producing the chimeric envelope polypeptide for targeting a retroviral vector to cells containing an Erb-B2 receptor, and is described in Batra, et al., Proc. Nat. Acad. Sci., Vol. 89, pgs. 5867-5871 (1992), was amplified by mixing 125ng of such DNA and 60pmoles each of AD275 and AD276 in a standard 100µl PCR reaction. The product was ethanol precipitated, resuspended and digested with Sfi I and Not I. The digest was electrophoresed through 1.0% Agarose and the digested product was excised from the gel and recovered by electroelution and ethanol precipitation. This fragment was ligated to pBSR(L) that had been digested with Sfi I and Not I. pBSR-e23 was isolated by screening for the presence of insert by digestion. It was sequenced in its entirety to ensure it was free of PCR introduced mutations.

II. Construction of backbone plasmids derived from pCAE and pCEE+

Single-strand pCAE (Morgan, *et al.*, 1993) was used as a template for site-directed mutagenesis using AD423 to remove the EcoRI site in pCAE (at bp 2,105) to form pCAE/RI. pCAE/RI was linearized by Not I, and then filled in with

Klenow fragment, and the plasmid was recircularized, thus forming pCAE/2#.

Single-stranded pCAE was used as a template, and site-directed mutagenesis was employed using oligonucleotides AD421 and AD422 to create AvrII (at bp 122) and MluI (at bp 354) sites, thereby forming pCAE/AM.

Both the pCAE/2# and CAE/AM were digested with XbaI, and the 3,866 bp fragment from CAE/2# was ligated with the 2,088 bp fragment from pCAE/AM, thus forming the backbone plasmid pCAE/3#. pCAE/3# was confirmed by restriction enzyme digestion and DNA sequencing.

Single-stranded pCEE+ was used as a template, and oligonucleotide AD604 was used in site-directed mutagenesis to create a Bgl II site in pCee+ (at bp 1385) to form pCee+/BglII.

Single-stranded pCee+ again was used as a template, and oligonucleotide AD605 was used in site-directed mutagenesis to create an EcoNI site in pCee+ (at bp 1568) to form pCee+/EcoNI.

pCee+/BglII and pCee+/EcoNI both were digested with BstXI, and the 2,021 bp fragment from pCee+/EcoNI was ligated to the 4,333 bp fragment from pCee+/BglII to form pCEE+/BN. pCee+/BN then was digested with Not I, filled in, and recircularized to form pCee+/BNΔN'.

Both pCee+ and pCee+/BNΔN' were digested by BstEII and BspEI, and the 586 bp fragment from pCee+/BNΔN' was ligated to the 5,768 bp fragment from pCee+ to form the backbone plasmid pCee+/BNΔN. The plasmid was identified by restriction enzyme digestion and confirmed by DNA sequencing.

III. Introduction of SfiI and Not I sites into envelope sequence.

By splicing overlap extension PCR, oligonucleotides AD870, AD871, AD422, and AD416 were used to introduce an SfiI site at the 5'-end of pCAE/3# (at bp 266). The PCR amplified

fragment was digested by AvrII and MluI and cloned into pCAE/3# digested with corresponding enzymes to form pA/S1. The plasmid was identified by Sfi I digestion and DNA sequencing.

pA/S1 was linearized by digestion with MluI and dephosphorylated. A phosphorylated double-stranded linker, made by heating and annealing AD889 and AD890 was ligated into this site to form A/S1N1. The clones were screened by PCR and sequenced.

By splicing overlap extension PCR, oligonucleotides AD887 , AD888, AD109 and AD111 were used to introduce NotI site at the 3'-end of A/S1 (corresponding to bp407 of CAE). The PCR amplified fragment was digested by AvrII and MluI and cloned into A/S1 digested with the corresponding enzymes to form A/S1N2. Plasmid was identified by SfiI and Not I digestion and DNA sequencing.

A/S1 was linearized by digestion with EcoRI and dephosphorylated. A phosphorylated double stranded linker, made by heating and annealing AD938 and AD939 was ligated into this site to form A/S1N3. Resultant clones were screened by PCR and sequenced.

Cee+/BNΔN was linearized by digestion with BstEII and dephosphorylated. A phosphorylated double stranded linker, made by heating and annealing AD936 and AD937 was ligated into this site to introduce an SfiI site at 1316bp of Cee+ to form E/SO. Resultant clones were screened by PCR and sequenced.

Cee+/BNΔN was linearized by digestion with BglII and dephosphorylated. A phosphorylated double stranded linker, made by heating and annealing AD868 and AD869 was ligated into this site to introduce an SfiI site at 1397bp of Cee+ to form E/S1. Resultant clones were screened by PCR and sequenced.

By splicing overlap extension PCR, oligos AD879, AD880, AD725 and AD287 were used to introduce an SfiI site at

the 5'-end of Cee+/BNΔN (1475bp). The PCR amplified fragment was digested by BglII and EcoNI and cloned into Cee+/BNΔN digested with the corresponding enzyme to form E/S2. Plasmid was identified by SfiI digestion and DNA sequencing.

By splicing overlap extension PCR, oligos AD881, AD882, AD15 and AD16 were used to introduce a NotI site at the 3'-end of E/S2 (1544bp). The PCR amplified fragment was digested by SfiI and BspEI and cloned into E/S2 digested with the corresponding enzyme to form E/S2N1. Plasmid was identified by SfiI and NotI digestion and DNA sequencing.

By splicing overlap extension PCR, oligos AD883, AD884, AD15 and AD16 were used to introduce a NotI site at the 3'-end of E/S1 (1610bp). The PCR amplified fragment was digested by SfiI and BspEI and cloned into E/S1 digested with the corresponding enzyme to form E/S1N2. Plasmid was identified by SfiI and NotI digestion and DNA sequencing.

By splicing overlap extension PCR, oligos AD885, AD886, AD15 and AD16 were used to introduce a NotI site at the 3'-end of E/S1 (1661bp). The PCR amplified fragment was digested by SfiI and BspEI and cloned into E/S1 digested with the corresponding enzyme to form E/S1N3. Plasmid was identified by SfiI and NotI digestion and DNA sequence.

IV. Construction of e23FV-env Chimeras into different expression vectors

A. Construction of e23FV-env chimeras into CAE/3# and Cee+/BNΔN derived vectors.

Chimeric envelope proteins were constructed by replacing a discrete segment of the envelope gene with the sequences encoding the e23Fv. The plasmid pBSR-e23 was digested with Sfi I and Not I, and a fragment containing the e23Fv sequences was isolated. Plasmids A/S1N1, A/S1N2, A/S1N3, E/S2N1, E/N1S2, and E/S1N3 were digested with Sfi I and Not I, and each digested plasmid was separately ligated to an aliquot of the e23Fv fragment to yield the chimeras named ChA1, ChA2, ChA3, ChE1, ChE2, and ChE3, respectively.

B. Construction of e23FV-env chimeras into LEESN (Ecotropic envelope protein was cloned into LXSN vector)

The plasmid LEESN was obtained from Jack Ragheb (NIH). This plasmid was constructed by digesting CEE (Morgan et al., 1993) with EcoRI and isolating the fragment encoding the Moloney Murine Leukemia Virus envelope protein. This fragment was ligated to LXSN (Genbank Accession #M28248) that had been digested with EcoRI. This plasmid was digested with ClaI and SalI to remove sequences extending from within the *env* cytoplasmic domain through the end of the 3'-untranslated sequences. These sequences were replaced with a double-stranded oligonucleotide encoding the Moloney *env* sequences from the ClaI site through the stop codons of the open reading frame, followed immediately by the nucleotide recognition sequence for the enzyme SalI. LEESN expresses RNA transcripts encoding the *env* sequences in the sense orientation.

The chimeras ChE1, ChE2 and ChE3 were each digested with EcoRI and ClaI and the fragment encoding e23-*env* was isolated. LEESN was digested with EcoRI and ClaI and the fragment encoding the LTRs and other vector sequences was ligated to the fragment derived from each of the chimeras (ChE1, ChE2, ChE3) to yield LChE1SN, LChE2SN, and LChE3SN. The CAE-derived chimeras (ChA1, ChA2, ChA3) were each digested with XbaI and incubated with Klenow to fill in the 5'-extension. Each plasmid was then digested with ClaI and the e23Fv-*env* encoding fragment was isolated. LEESN was digested with EcoRI, incubated with Klenow, and subsequently digested with ClaI. The fragment containing the retroviral vector sequences of LEESN was ligated to the isolated fragments from ChA1, ChA2, and ChA3 to yield respectively LChA1SN, LChA2SN, and LChA3SN.

C. Transfection of Psi-2 cells

Psi-2 cells (obtained from ATCC) were grown in D10 medium (Dulbecco's Modified Eagles' Medium with 4,500 g/l

glucose and sodium pyruvate, supplemented with 10% FCS and 2 mM glutamine). The cells were plated at a concentration of 6 x 10^5 cells per 100 mm plate, and cultured for 16-24 hours. The medium was replaced 4 hours prior to transfection. The cells were transfected with LChA1SN, LChA2SN, LChA3SN, LChE1SN, LChE2SN, or LChE3SN. Calcium phosphate precipitate was made using a DNA Transfection Kit (5' → 3'; Boulder, Colorado). For stable transfections, D10 containing precipitate was replaced for 8 hours, at which time the medium was changed to the appropriate selective media containing D10 and G418 at 0.8 mg/ml. Selection with G418 continued for 8-10 days.

D. Collection and processing of tissue culture supernatant

The medium (containing D10 and G418) was pipetted from transfected cell monolayers of IV.C. above and filtered through 0.45μm syringe filters (Millipore) and stored at -70°C. In order to concentrate the collected retroviral supernatant, the supernatant was spun at 4°C in centriprep 100 ultra-filtration units (Amicon) at 450 xg until the desired volume was attained.

E. FACS binding assay

The supernatants of IV.D. above obtained through transfection of LChA1SN, LChA2SN, LChA3SN, LChE1SN, LChE2SN, and LChE3SN (IV.C. above) were subjected to an FACS binding assay to measure e23-mediated binding to T47D or SK-BR-3 cells. T47D cells (obtained from ATCC) are human breast cells which have high levels of expression of the Erb-B2 receptor. Sk-BR-3 cells (obtained from ATCC) have lower binding affinity to Erb-B2 monoclonal antibody. Prior to the assay, T47D cells were grown in D10 and F12 (supplemented with 10% FCS and 2mM glutamine) in the ratio of 1:1. SK-BR-3 cells were grown in Mc10 (McCoy's 5A medium supplemented with 10% FCS and 2 mM glutamine).

e23-mediated binding to cells was measured using a derivative of the method of Kadan, *et al.*, J. Virol., Vol. 66, No. 4, pgs. 2281-2287 (1992). T47D and SK-BR-3 cells were suspended as follows: Monolayers were rinsed in PBS (Gibco) and incubated in Enzyme Free Cell Dissociation Buffer (Gibco) for 10 minutes at room temperature. Cells were removed from the plate by vigorous agitation. Cell suspensions were triturated briefly with a 1 ml micropipet, and diluted with cell growing media to 10 ml per plate. The cells then filtered through a 50 um cell strainer (Falcon), and counted using a hemocytometer. Cells were aliquoted at 2 x 10^5 cells per tube and collected by centrifugation. Cells were resuspended in retroviral supernatant and incubated at room temperature for 1 hour. Cells were collected by centrifugation for 6 seconds and washed once in PBS with 10% goat serum. Cells were resuspended in monoclonal antibody 83A25 directed against C-terminal of gp70 (Evans, *et al.*, J. Virol., Vol. 64, No. 12, pgs. 6176-6183 (1990)) and incubated for 1 hour at 4°C. Cells were collected by centrifugation and washed 3 times in PBS with 10% goat serum. Cells were resuspended in FITC-conjugated goat-anti-rat antisera (Kirkegaard) and incubated for 30 minutes at 4°C. Following one wash in PBS with 10% goat serum, the cells were resuspended in 4% paraformaldehyde and analyzed by flow cytometry. The FACS data, as indicated by mean channel shift, which indicates the degree of binding of the retrovirus to the targeted cells, is summarized in Table 1 hereinbelow.

F. GP8 cell surface expression assay

The viral supernatants from IV.D. above also were employed in a GP8 cell surface expression assay. Prior to such assay, GP8 cells (Morgan, et al., 1993) were grown in D10 medium.

The supernatants were mixed with GP8 cells with 8ug/ml of Polybrene and left for 16-24 hours to provide for

transduction of the GP8 cells. The media then was changed to D10 with 0.8mg/ml G418. 8-10 days later, cells were resuspended with Enzyme Free Cell Dissociation Buffer as described above. Cells were aliquoted at 2 x 10^5 cells per tube and collected by centrifugation. Cells were resuspended in monoclonal antibody 83A25 and were processed and analyzed further as in the binding assay described above. Assay results are given in Table 1 hereinbelow.

G. Titer in NIH3T3

The viral supernatants of IV.D. above also were used in an NIH3T3 cell titer assay. 3 x 10^4 NIH3T3 cells were plated to each of the wells in a 6 well titer plate in D10 medium. 12-24 hours later, supernatant from transfection of Psi-2 cells were added to the plate with the Polybrene at a concentration of 8ug/ml. 12-16 hours later, the media was changed to D10/G418 (0.8mg/ml). Cells were kept in this selection medium for 8-10 days and then stained with 1% Methylene blue in methanol and then counting stained colonies. The assay results are given in Table 1 below.

Table 1

Virus (LChSN-->Psi2)	Mean Channel Shift T47D	SKBr3	3T3 Titer (x 10^6)	GP8 surface expression
LEESN	11.28	9.99	3.0	++++
LChA1SN	25.01	11.59	6.0	++
LChA2SN	16.86	11.53	7.7	+++
LChA3SN	14.31	11.04	5.5	+++
LChE1SN	14.95	10.36	6.4	+++
LChE2SN	12.99	12.06	10.0	+++
LChE3SN	13.64	9 .72	6.2	+++

H. Construction of e23-env chimeras into pRSV-1 vector

pRSV-1 is a plasmid which contains wild type mouse DHFR cDNA driven by the SV40 promoter, an SV40 poly A sequence, an ampicillin resistance gene, and in the 5' end of the polylinker, there is an RSV LTR promoter. pRSV-1 also is described in Kohli, et al., J.Cell.Physiol., Vol. 142, pgs. 194-200 (1990). pRSV-1 plasmid was linearized by EcoRV at a polylinker region and dephosphorylated . The e23FV-env chimeras in Cee+ based backbone (ChE1, ChE2, and ChE3) were digested with EcoRI, and chimeras in CAE based backbone (ChA1, ChA2 and ChA3) were digested with XbaI and EcoRI. Then the e23FV-env fragments were filled in by Klenow and ligated into the pRSV-1 vector. The resultant plasmids were identified by enzyme digestion and named as ChA1RSV, ChA2RSV, ChA3RSV, ChE1RSV, ChE2RSV, and ChE3RSV. Chimeras in pRSV-1 are transfected into GPNZ cells (Morgan, et al., 1993), selected with MTX and amplified. Positive expressing clones are identified by e23 cDNA, PCR priming and immunostaining. Alternatively, chimeras in pRSV-1 are transfected into Psi-2

cells, and subjected to MTX selection and amplification. The cells may be transfected with an appropriate retroviral plasmid vector to produce targeted retroviral particles.

Example 2

The approach of this example was to replace small, disulfide-constrained segments of the ecotropic Moloney murine leukemia virus envelope protein with other receptor-specific ligands. Ideally, the disulfide-constrained form of the ligands should bind to their specific receptors as well as or better than their corresponding linear form. A constrained form of α-melanotropin stimulating hormone (α-MSH) has been chosen to test this approach. This constrained peptide hormone binds to the target receptors on melanoma cells with at least as high an affinity as the linear form of α-MSH, and in biological assays has a greater detectable binding than the linear form. There are several advantages to using α-MSH as a ligand. One advantage is that there is a simple biological assay system which is very sensitive at detecting ligand binding to receptor. Some human and mouse melanoma tumor cell lines have been found to secrete melanin when stimulated by binding of α-MSH ligand to the cell-surface MSH receptors. This melanin production can be quantitatively detected using a spectrophotometer which reads an absorbance at 405mm. In addition to the above reasons for choosing α-MSH as the ligand, MSH receptors are present on many melanomas, and the ability to target these tumors with a gene therapy vector may allow for successful treatment of this type of cancer which is rapidly increasing in prevalence.

Construction of pcDNA-EF

The plasmid pcDNA-EF is a result of several manipulations of the Moloney murine leukemia virus (MoMuLV) envelope sequence beginning with the plasmid pCEE (Morgan, *et al.*, 1993). Plasmid pBB2-E, was constructed by first amplifying by PCR the coding sequence for the first 262 amino

acids of the MoMuLV ecotropic envelope gp 70 protein (i.e., 33aa signal sequence and the first 229 amino acids (i.e., (SEQ ID NO:1)) of the mature protein) from pCEE. The oligonucleotides used in this construction were:

oligo71 5'-GGAGCTAGCTAGACTGACATGGCGCGTTC-3'

oligo72 5'-CTGTGATCACTATAGATTTTGGTATCTGAGTCG-3'

This PCR product was digested with NheI and BclI and cloned into the NheI and BamHI sites of the plasmid pBlueBac2 (Invitrogen Corp., San Diego, CA) to form pBB2-E. The plasmid was modified further by linearizing pBB2-E with BamHI (which cuts at amino acid #222) and inserting two hybridized oligonucleotides (AD298 and AD299) to regenerate amino acids 222-229 as well as a convenient C-terminal fused "FLAG" epitope tag (Kodak/IBI, Rochester, NY) which can be used for facilitating protein purification of this 229 amino acid mature protein. Primers AD298 and AD299 have the following sequences:

AD298 5'-GATCAGGCTCAGATACCAAAATCTAGACTACAAGGAC
GACGATGACAAGTAG-3'

AD299 5'-GATCCTACTTGTCATCGTCGTCCTTGTAGTCTAGATTT
TGGTATCTGAGCCT-3'

After obtaining the modified plasmid, pBB2-EF, this plasmid was then digested with HpaI and BamHI, to remove the entire coding domain of (SEQ ID NO:1) including the "FLAG" tag, and such fragment was cloned into pCEE+ to construct the intermediary construct pCEE+F. To create the final plasmid pcDNA-EF, pCEE+F was digested with EcoRI and NotI to remove the entire coding region of (SEQ ID NO:1)/FLAG tag fusion protein and such fragment was cloned into EcoRI and NotI sites of pcDNA3 (Invitrogen Corp., San Diego, CA), thereby generating pcDNA-EF.

Construction of Alpha-Melanotropin Stimulating Hormone/Murine Leukemia Virus Chimeric Envelope Proteins

The α-Melanotropin Stimulating Hormone (alpha-MSH)/MuLV chimeric proteins were constructed using overlapping PCR Mutagenesis (Ho, S.N., *et al.* (1989) Gene 77, 51-59) to insert/replace either amino acids S74-S91 or G80-P88 in the coding region for (SEQ ID NO:1) of pcDNA-EF. The oligonucleotides used in the construction of these alpha-MSH/MuLV chimeric proteins and the location of replaced amino acids are indicated below:

Name	Sequence (5'-->3')	Description
AD15	ATGTAAGCCCTGGATCTTGTCCGG	oligo for PCR screening and sequencing
AD16	ATGGGCAACTTCTGGCAACCACCC	same as above
AD738	CACTTCGGACAGGGTCAACTTG TGTTGG	oligo for overlapping PCR mutagenesis
AD740	GACTAAGAACCTAGAACCTCGCTGG	same as above
AD836	GTAATACGACTCACTATAGGGC	oligo for PCR screening and sequencing
AD837	ATTTAGGTGACACTATA	same as above
AF39	TGGTGCAAGGCTGTTTGCG(C or A)ACATTTTCGATGGGGTAAGGCCCTCACCC CTCGGTGCAAC	oligo for amino acid replacement of residues S74-S91 of (SEQ ID NO:1)
AF40	GCAAACAGCCTTGCACCATCTGA AGTGT(G or T)CGCAACAAGGGGGCC CCGG	bottom strand complement of AF39
AF44	CATTTCCGATGGTGCAAGCCGGT ATTAACCTCCCTCACCCCTCG	oligo for amino acid replacement of resideues G80-P88 of (SEQ ID NO:1)
AF45	CTTGCACCATCGGAAATGT(T or G)CACAGCTTGGGCTGCTGCC	bottom strand complement of AF44

Initial PCR reactions involved the amplification of primer combinations AD740+AF40, AD740+AF45, AD738+AF39, and AD738+AF44 in individual reactions consisting of PCR buffer (final concentration: 50mM KCl, 10mM Tris-HCl, pH8.3, 1.5mM $MgCl_2$, and 0.001% gelatin), 0.2mM dNTPs, 80mM additional $MgCl_2$, 0.5 units Taq polymerase, 50 ng of pcDNA-EF plasmid, and 12.5pmol of each oligo/25μl reaction (all buffers, $MgCl_2$, and Taq polymerase obtained from Perkin Elmer). The reactions were initially denatured at 95°C for 2 minutes, and then cycled at 95°C x 15 seconds, 50°C x 30 seconds, and

72°C x 15 seconds for 35 cycles in a Perkin-Elmer 9600 Tempcycler. PCR products were identified and purified from 2.5% agarose (2% NuSieve, 0.5% SeaKem; FMC, Rockland, Maine) gels using NA45 DEAE ion exchange paper (Millipore, Bedford, MA). After this initial amplification, the PCR products were joined [(AD740+AF40)+(AD738+AF39) and (AD740+AF45)+(AD738+AF44)] and amplified in reactions similar to that detailed above, except no pcDNA-EF plasmid was added to the reactions, and the only oligonucleotides added to the reactions were AD738 and AD740 at the above indicated concentrations. After this second amplification, a small portion of the PCR products were again identified using agarose gel electrophoresis/ethidium bromide staining. The remainder of the PCR products were extracted with phenol/chloroform/isoamyl alcohol (25:24:1), back extracted with 1 mM Tris-HCl (pH8.0)/1 mM EDTA, and the aqueous phase precipitated by addition of 0.1 volume of 3M sodium acetate, pH 4.6 and 2.5 volumes of 100% ethanol. After removing the polymerase used in the PCR reaction by extraction and precipitation, the PCR products were digested with restriction enzymes BstEII (New England Biolabs, Beverly, MA) and AccIII (Promega, Madison, WI), purified using agarose gel electrophoresis as above, and subsequently ligated into the BstEII and AccIII sites of the pcDNA-EF plasmid. Frozen competent XL1-blue E. coli (Stratagene, La Jolla, California) were transformed with the ligation products according to the method of Hanahan, J. Mol. Biol., Vol. 166, pgs. 557-580 (1983). Transformed colonies were screened by PCR amplification using oligonucleotide primers (AD15 and AD16) which amplified the entire alpha-MSH/MuLV PCR insert. The PCR products were then sequenced by PCR sequencing (AmpliTaq Cycle Sequencing: Perkin Elmer, Foster City, CA). The amino acid replacement plasmids are p3-1 and p3-2, and replace residues S74-S91 with the residues A-H-F-R-W-C-K-A-V-C-E-H-F-R-W-G-K-A and E-H-F-R-W-C-K-A-V-C-E-H-F-R-W-G-K-A,

respectively. The amino acid replacement plasmid p6-3 replaces residues G80-P88 with S-C-A-H-F-R-W-C-K-P-V.

Transfection and Collection of Chimeric AlphaMSH/MuLV Ecotropic gp 70 protein

COS7 cells were maintained in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum and 2mM glutamine at 37°C and 5% CO_2. Plasmid DNA from p3-1, p3-2, or p6-3 was prepared for transfection from overnight cultures with a Plasmid Maxi Kit (Qiagen, Chatsworth, CA). COS7 cells were plated on 100mm tissue culture dishes at a concentration of 6×10^5 cells/100mm dish. Twelve to sixteen hours after plating the cells, the media in the dish was aspirated and 9ml of fresh media was added to the cells. The cells were then returned to the incubator at 37°C/5% CO_2. After an additional 2-4 hours, the cells were transfected with 30μg plasmid DNA by the calcium phosphate method (Graham, F.L. and A.J. van der Eb (1973) Virology 52, 456-467). Approximately 12-16 hours after transfection of the COS7 cells, the media was aspirated, cells were washed with 10ml of Dulbecco's phosphate-buffered saline, and fresh media was added to the cells. Approximately 48 hours after transfection, the media was aspirated from the plates and replaced with DMEM + 1% fetal bovine serum + 2 mM glutamine containing 600 μg/ml G418 for selection of COS7 cells. Selection of cells continued for two weeks until all cells not expressing neomycin phosphotransferase were killed. For the biological assays described below, the supernatants containing the αMSH/MuLV chimeric proteins were collected for three days in DMEM + 1% fetal bovine serum + 2 mM glutamine and filtered through a 0.45μ Millex-HA membrane (Millipore, Bedford, MA).

Biological Assay to Determine Binding of Chimeric Protein

B16-F1 mouse melanoma cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10%

fetal bovine serum and 2mM glutamine at 37°C and 5% CO_2. These B16-F1 cells secrete melanin when stimulated by the binding of alpha-MSH to the cell-surface receptors. The melanin which is secreted can be measured spectrophotometrically at an absorbance of 405nm. The B16-F1 cells were plated in a 96-well microtiter plate at a concentration of 2500 cells/well. Approximately 24 hours after plating the cells, the media was aspirated and either media which contained known concentrations of alpha-MSH ligand (Sigma, St. Louis, MO) or media collected from the above stably transfected COS7 cells was added to the B16-F1 cells. Three to four days after the ligand or COS7 conditioned media was added to the B16-F1 cells, the samples were analyzed on a Dynatech MR700 (Dynatech, Chantilly, Virginia) microtiter plate spectrophotometer at a wavelength of 405nm.

Previous studies established that endogenous proteins in the fetal bovine serum stimulated melanin production from B16-F1 mouse melanoma cells, and therefore resulted in a significant background which affected the biological binding assay. The data shown in Figure 3 is expressed in percentage relative to the α-MSH ligand (100%), after the background (media without ligand) is subtracted. As shown in Figure 3, media from cells which expressed (SEQ ID NO:1) alone showed a 9.3% increase in melanin production, but media from cells which expressed the α-MSH/MuLV protein stimulated melanin production from 26.6% to 29.7%. This increase in melanin production illustrates the binding of the chimeric proteins to the MSH receptors on the B16-F1 melanoma cells. This experiment was repeated and similar results were observed.

Example 3

Generation of Retroviral Producer Cell Line Incorporating the α-MSH/gp70 Envelope Protein

Each of plasmids p3-1, p3-2, and p6-3 is digested with BstEII and AccIII, and the resulting fragment encoding the

chimeric protein is cloned into BstEII and AccIII digested pCEE+.

After confirming the presence of the αMSH sequence in the pCEE+ plasmids, these plasmids are prepared for transfection into the pre-producer cell line, GPL. The chimeric αMSH/MuLV envelope protein plasmids are cotransfected with the plasmid pPUR (Clonetech, Palo Alto, CA) at a ratio of 29:1 to allow for selection of stable producer clones with the antibiotic puromycin. After selection of stable clones, the best producer clone is identified by screening for envelope protein expression on the cell surface, as well as virus binding, fusion, and transduction of MSH-specific target cells.

The disclosure of all patents, publications (including published patent applications), and database entries referenced in this application are specifically incorporated herein by reference in their entirety to the same extent as if each such individual patent, publication, and database entry were specifically and individually indicated to be incorporated by reference.

It is to be understood, however, that the scope of the present invention is not to be limited to the specific embodiments described above. The invention may be practiced other than as particularly described and still be within the scope of the accompanying claims.

SEQUENCE LISTING

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(B) COMPUTER: IBM PS/2
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(D) SOFTWARE: Word Perfect 5.1

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(2) INFORMATION FOR SEQ ID NO: 1:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 229 amino acids
(B) TYPE: amino acids
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(ix) FEATURE:
(A) NAME/KEY: Receptor binding region of ecotropic gp70 protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Ala Ser Pro Gly Ser Ser Pro
5
His Gln Val Tyr Asn Ile Thr Trp Glu Val
10 15
Thr Asn Gly Asp Arg Glu Thr Val Trp Ala
20 25
Thr Ser Gly Asn His Pro Leu Trp Thr Trp
30 35
Trp Pro Asp Leu Thr Pro Asp Leu Cys Met
40 45
Leu Ala His His Gly Pro Ser Tyr Trp Gly

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          50                  55
Leu Glu Tyr Gln Ser Pro Phe Ser Ser Pro
          60                  65
Pro Gly Pro Pro Cys Cys Ser Gly Gly Ser
          70                  75
Ser Pro Gly Cys Ser Arg Asp Cys Glu Glu
          80                  85
Pro Leu Thr Ser Leu Thr Pro Arg Cys Asn
          90                  95
Thr Ala Trp Asn Arg Leu Lys Leu Asp Gln
         100                 105
Thr Thr His Lys Ser Asn Glu Gly Phe Tyr
         110                 115
Val Cys Pro Gly Pro His Arg Pro Arg Glu
         120                 125
Ser Lys Ser Cys Gly Gly Pro Asp Ser Phe
         130                 135
Tyr Cys Ala Tyr Trp Gly Cys Glu Thr Thr
         140                 145
Gly Arg Ala Tyr Trp Lys Pro Ser Ser Ser
         150                 155
Trp Asp Phe Ile Thr Val Asn Asn Asn Leu
         160                 165
Thr Ser Asp Gln Ala Val Gln Val Cys Lys
         170                 175
Asp Asn Lys Trp Cys Asn Pro Leu Val Ile
         180                 185
Arg Phe Thr Asp Ala Gly Arg Arg Val Thr
         190                 195
Ser Trp Thr Thr Gly His Tyr Trp Gly Leu
         200                 205
Arg Leu Tyr Val Ser Gly Gln Asp Pro Gly
         210                 215
Leu Thr Phe Gly Ile Arg Leu Arg Tyr Gln
         220                 225
```

Asn Leu

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 209 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY: Receptor binding region of amphotropic gp 70 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Val Gly Met Ala Glu Ser Pro His Gln Val
5 10
Phe Asn Val Thr Trp Arg Val Thr Asn Leu
15 20
Met Thr Gly Arg Thr Ala Asn Ala Thr Ser
25 30
Leu Leu Gly Thr Val Gln Asp Ala Phe Pro
35 40
Lys Leu Tyr Phe Asp Leu Cys Asp Leu Val
45 50
Gly Glu Glu Trp Asp Pro Ser Asp Gln Glu
55 60
Pro Tyr Val Gly Tyr Gly Cys Lys Tyr Pro
65 70
Ala Gly Arg Gln Arg Thr Arg Thr Phe Asp
75 80
Phe Tyr Val Cys Pro Gly His Thr Val Lys
85 90
Ser Gly Cys Gly Gly Pro Gly Glu Gly Tyr
95 100
Cys Gly Lys Trp Gly Cys Glu Thr Thr Gly
105 110
Gln Ala Tyr Trp Lys Pro Thr Ser Ser Trp

115 120
Asp Leu Ile Ser Leu Lys Arg Gly Asn Thr
125 130
Pro Trp Asp Thr Gly Cys Ser Lys Val Ala
135 140
Cys Gly Pro Cys Tyr Asp Leu Ser Lys Val
145 150
Ser Asn Ser Phe Gln Gly Ala Thr Arg Gly
155 160
Gly Arg Cys Asn Pro Leu Val Leu Glu Phe
165 170
Thr Asp Ala Gly Lys Lys Ala Asn Trp Asp
175 180
Gly Pro Lys Ser Trp Gly Leu Arg Leu Tyr
185 190
Arg Thr Gly Thr Asp Pro Ile Thr Met Phe
195 200
Ser Leu Thr Arg Gln Val Leu Asn Val
205

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 209 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY: Receptor binding region of 10A1 murine leukemia virus envelope

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Val Gly Met Ala Glu Ser Pro His Gln Val
5 10
Phe Asn Val Thr Trp Arg Val Thr Asn Leu
15 20

Met Thr Gly Arg Thr Ala Asn Ala Thr Ser
25 30
Leu Leu Gly Thr Val Gln Asp Ala Phe Pro
35 40
Arg Leu Tyr Phe Asp Leu Cys Asp Leu Val
45 50
Gly Glu Glu Trp Asp Pro Ser Asp Gln Glu
55 60
Pro Tyr Val Gly Tyr Gly Cys Lys Tyr Pro
65 70
Gly Gly Arg Lys Arg Thr Arg Thr Phe Asp
75 80
Phe Tyr Val Cys Pro Gly His Thr Val Lys
85 90
Ser Gly Cys Gly Gly Pro Arg Glu Gly Tyr
95 100
Cys Gly Glu Trp Gly Cys Glu Thr Thr Gly
105 110
Gln Ala Tyr Trp Lys Pro Thr Ser Ser Trp
115 120
Asp Leu Ile Ser Leu Lys Arg Gly Asn Thr
125 130
Pro Trp Asp Thr Gly Cys Ser Lys Met Ala
135 140
Cys Gly Pro Cys Tyr Asp Leu Ser Lys Val
145 150
Ser Asn Ser Phe Gln Gly Ala Thr Arg Gly
155 160
Gly Arg Cys Asn Pro Leu Val Leu Glu Phe
165 170
Thr Asp Ala Gly Lys Lys Ala Asn Trp Asp
175 180
Gly Pro Lys Ser Trp Gly Leu Arg Leu Tyr
185 190
Arg Thr Gly Thr Asp Pro Ile Thr Met Phe

195 200

Ser Leu Thr Arg Gln Val Leu Asn Ile

205

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 201 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS:

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polypeptide

(ix) FEATURE:

(A) NAME/KEY: Receptor binding region of xenotropic murine leukemia virus.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Ala Ser Val Gln Arg Asp Ser Pro His Gln

5 10

Ile Phe Asn Val Thr Trp Arg Val Thr Asn

15 20

Leu Met Thr Gly Gln Thr Ala Asn Ala Thr

25 30

Ser Leu Leu Gly Thr Met Thr Asp Thr Phe

35 40

Pro Lys Leu Tyr Phe Asp Leu Cys Asp Leu

45 50

Val Gly Asp Tyr Trp Asp Asp Pro Glu Pro

55 60

Asp Ile Gly Asp Gly Cys Arg Thr Pro Gly

65 70

Gly Arg Arg Arg Thr Arg Leu Tyr Asp Phe

75 80

Tyr Val Cys Pro Gly His Thr Val Pro Ile

85 90

Gly Cys Gly Gly Pro Gly Glu Gly Tyr Cys

95 100

Gly Lys Trp Gly Cys Glu Thr Thr Gly Gln
105 110
Ala Tyr Trp Lys Pro Ser Ser Ser Trp Asp
115 120
Leu Ile Ser Leu Lys Arg Gly Asn Thr Pro
125 130
Lys Asp Gln Gly Pro Cys Tyr Asp Ser Ser
135 140
Val Ser Ser Gly Val Gln Gly Ala Thr Pro
145 150
Gly Gly Arg Cys Asn Pro Leu Val Leu Glu
155 160
Phe Thr Asp Ala Gly Arg Lys Ala Ser Trp
165 170
Asp Ala Pro Lys Val Trp Gly Leu Arg Leu
175 180
Tyr Arg Ser Thr Gly Ala Asp Pro Val Thr
185 190
Arg Phe Ser Leu Thr Arg Gln Val Leu Asn
195 200
Val

(2) INFORMATION FOR SEQ ID NO: 5:
(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 198 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS:
(D) TOPOLOGY: linear
(ii) MOLECULE TYPE: polypeptide
(ix) FEATURE:
(A) NAME/KEY: Receptor binding region of polytropic MX27 provirus.
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
Val Ser Val Gln His Asp Ser Pro His Gln
5 10

Val Phe Asn Val Thr Trp Arg Val Thr Asn
15 20
Leu Met Thr Gly Gln Thr Ala Asn Ala Thr
25 30
Ser Leu Leu Gly Thr Met Thr Asp Ala Phe
35 40
Pro Lys Leu Tyr Phe Asp Leu Cys Asp Leu
45 50
Ile Gly Asp Asp Trp Asp Glu Thr Gly Leu
55 60
Gly Cys Arg Thr Pro Gly Gly Arg Lys Arg
65 70
Ala Arg Thr Phe Asp Phe Tyr Val Cys Pro
75 80
Gly His Thr Val Pro Thr Gly Cys Gly Gly
85 90
Pro Arg Glu Gly Tyr Cys Gly Lys Trp Gly
95 100
Cys Glu Thr Thr Gly Gln Ala Tyr Trp Lys
105 110
Pro Ser Ser Ser Trp Asp Leu Ile Ser Leu
115 120
Lys Arg Gly Asn Thr Pro Arg Asn Gln Gly
125 130
Pro Cys Tyr Asp Ser Ser Ala Val Ser Ser
135 140
Asp Ile Lys Gly Ala Thr Pro Gly Gly Arg
145 150
Cys Asn Pro Leu Val Leu Glu Phe Thr Asp
155 160
Ala Gly Lys Lys Ala Ser Trp Asp Gly Pro
165 170
Lys Val Trp Gly Leu Arg Leu Tyr Arg Ser
175 180
Thr Gly Thr Asp Pro Val Thr Arg Phe Ser

185 190

Leu Thr Arg Gln Val Leu Asn Ile

195

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 687 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(ix) FEATURE:

(A) NAME/KEY: polynucleotide encoding receptor binding region of ecotropic gp 70 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

GCT TCG CCC GGC TCC AGT CCT CAT CAA GTC

TAT AAT ATC ACC TGG GAG GTA ACC AAT GGA 60

GAT CGG GAG ACG GTA TGG GCA ACT TCT GGC

AAC CAC CCT CTG TGG ACC TGG TGG CCT GAC 120

CTT ACC CCA GAT TTA TGT ATG TTA GCC CAC

CAT GGA CCA TCT TAT TGG GGG CTA GAA TAT 180

CAA TCC CCT TTT TCT TCT CCC CCG GGG CCC

CCT TGT TGC TCA GGG GCC AGC AGC CCA GGC 240

TGT TCC AGA GAC TGC GAA GAA CCT TTA ACC

TCC CTC ACC CCT CGG TGC AAC ACT GCC TGG 300

AAC AGA CTC AAG CTA GAC CAG ACA ACT CAT

AAA TCA AAT GAG GGA TTT TAT GTT TGC CCC 360

GGG CCC CAC CGC CCC CGA GAA TCC AAG TCA

TGT GGG GGT CCA GAC TCC TTC TAC TGT GCC 420

TAT TGG GGC TGT GAG ACA ACC GGT AGA GCT

TAC TGG AAG CCC TCC TCA TCA TGG GAT TTC 480

ATC ACA GTA AAC AAC AAT CTC ACC TCT GAC

CAG GCT GTC CAG GTA TGC AAA GAT AAT AAG 540

TGG TGC AAC CCC TTA GTT ATT CGG TTT ACA

GAC GCC GGG AGA CGG GTT ACT TCC TGG ACC 600

ACA GGA CAT TAC TGG GGC TTA CGT TTG TAT

GTC TCC GGA CAA GAT CCA GGG CTT ACA TTT 660

GGG ATC CGA CTC AGA TAC CAA AAT CTA 687

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 627 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(ix) FEATURE:
(A) NAME/KEY: polynucleotide encoding receptor binding region of amphotropic gp 70 protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

GTA GGG ATG GCA GAG AGC CCC CAT CAG GTC

TTT AAT GTA ACC TGG AGA GTC ACC AAC CTG 60

ATG ACT GGG CGT ACC GCC AAT GCC ACC TCC

CTC CTG GGA ACT GTA CAA GAT GCC TTC CCA 120

AAA TTA TAT TTT GAT CTA TGT GAT CTG GTC

GGA GAG GAG TGG GAC CCT TCA GAC CAG GAA 180

CCG TAT GTC GGG TAT GGC TGC AAG TAC CCC

GCA GGG AGA CAG CGG ACC CGG ACT TTT GAC 240

TTT TAC GTG TGC CCT GGG CAT ACC GTA AAG

TCG GGG TGT GGG GGA CCA GGA GAG GGC TAC 300

TGT GGT AAA TGG GGG TGT GAA ACC ACC GGA

CAG GCT TAC TGG AAG CCC ACA TCA TCG TGG 360

GAC CTA ATC TCC CTT AAG CGC GGT AAC ACC

CCC TGG GAC ACG GGA TGC TCT AAA GTT GCC 420

TGT GGC CCC TGC TAC GAC CTC TCC AAA GTA

TCC AAT TCC TTC CAA GGG GCT ACT CGA GGG 480

GGC AGA TGC AAC CCT CTA GTC CTA GAA TTC

ACT GAT GCA GGA AAA AAG GCT AAC TGG GAC 540

GGG CCC AAA TCG TGG GGA CTG AGA CTG TAC

CGG ACA GGA ACA GAT CCT ATT ACC ATG TTC 600

TCC CTG ACC CGG CAG GTC CTT AAT GTG 627

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 627 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(ix) FEATURE:

(A) NAME/KEY: polynucleotide encoding receptor binding region of 10A1 murine leukemia virus envelope

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

GTA GGG ATG GCA GAG AGC CCC CAT CAG GTC

TTT AAT GTA ACC TGG AGA GTC ACC AAC CTG 60

ATG ACT GGG CGT ACC GCC AAT GCC ACC TCC

CTT TTA GGA ACT GTA CAA GAT GCC TTC CCA 120

AGA TTA TAT TTT GAT CTA TGT GAT CTG GTC

GGA GAA GAG TGG GAC CCT TCA GAC CAG GAA 180

CCA TAT GTC GGG TAT GGC TGC AAA TAC CCC

GGA GGG AGA AAG CGG ACC CGG ACT TTT GAC 240

TTT TAC GTG TGC CCT GGG CAT ACC GTA AAA

TCG GGG TGT GGG GGG CCA AGA GAG GGC TAC 300

TGT GGT GAA TGG GGT TGT GAA ACC ACC GGA

CAG GCT TAC TGG AAG CCC ACA TCA TCA TGG 360

GAC CTA ATC TCC CTT AAG CGC GGT AAC ACC

CCC TGG GAC ACG GGA TGC TCC AAA ATG GCT 420

TGT GGC CCC TGC TAC GAC CTC TCC AAA GTA

TCC AAT TCC TTC CAA GGG GCT ACT CGA GGG 480

GGC AGA TGC AAC CCT CTA GTC CTA GAA TTC

ACT GAT GCA GGA AAA AAG GCT AAT TGG GAC 540

GGG CCC AAA TCG TGG GGA CTG AGA CTG TAC

CGG ACA GGA ACA GAT CCT ATT ACC ATG TTC 600

TCC CTG ACC CGC CAG GTC CTC AAT ATA 627

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 603 bases

(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(ix) FEATURE:
(A) NAME/KEY: polynucleotide encoding receptor binding region of xenotropic murine leukemia virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GCC TCG GTA CAA CGT GAC AGC CCT CAC CAG

ATC TTC AAT GTT ACT TGG AGA GTT ACC AAC 60

CTA ATG ACA GGA CAA ACA GCT AAC GCC ACC

TCC CTC CTG GGG ACG ATG ACA GAC ACC TTC 120

CCT AAA CTA TAT TTT GAC CTG TGT GAT TTA

GTA GGA GAC TAC TGG GAT GAC CCA GAA CCC 180

GAT ATT GGG GAT GGT TGC CGC ACT CCC GGG

GGA AGA AGA AGG ACA AGA CTG TAT GAC TTC 240

TAT GTT TGC CCC GGT CAT ACT GTA CCA ATA

GGG TGT GGA GGG CCG GGA GAG GGC TAC TGT 300

GGC AAA TGG GGA TGT GAG ACC ACT GGA CAG

GCA TAC TGG AAG CCA TCA TCA TCA TGG GAC 360

CTA ATT TCC CTT AAG CGA GGA AAC ACT CCT

AAG GAT CAG GGC CCC TGT TAT GAT TCC TCG 420

GTC TCC AGT GGC GTC CAG GGT GCC ACA CCG

GGG GGT CGA TGC AAC CCC CTG GTC TTA GAA 480

TTC ACT GAC GCG GGT AGA AAG GCC AGC TGG

GAT GCC CCC AAA GTT TGG GGA CTA AGA CTC 540

TAT CGA TCC ACA GGG GCC GAC CCG GTG ACC

CGG TTC TCT TTG ACC CGC CAG GTC CTC AAT 600

GTA 603

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 594 bases

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: polynucleotide

(ix) FEATURE:

(A) NAME/KEY: polynucleotide encoding receptor binding region of polytropic MX 27 provirus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

GTA TCA GTA CAA CAT GAC AGC CCT CAT CAG

GTC TTC AAT GTT ACT TGG AGA GTT ACC AAC 60

TTA ATG ACA GGA CAA ACA GCT AAT GCT ACC

TCC CTC CTG GGG ACA ATG ACC GAT GCC TTT 120

CCT AAA CTG TAC TTT GAC TTG TGC GAT TTA

ATA GGG GAC GAC TGG GAT GAG ACT GGA CTC 180

GGG TGT CGC ACT CCC GGG GGA AGA AAA AGG

GCA AGA ACA TTT GAC TTC TAT GTT TGC CCC 240

GGG CAT ACT GTA CCA ACA GGG TGT GGA GGG

CCG AGA GAG GGC TAC TGT GGC AAA TGG GGC 300

TGT GAG ACC ACT GGA CAG GCA TAC TGG AAG

CCA TCA TCA TCA TGG GAC CTA ATT TCC CTT 360

AAG CGA GGA AAC ACC CCT CGG AAT CAG GGC

CCC TGT TAT GAT TCC TCA GCG GTC TCC AGT 420

GAC ATC AAG GGC GCC ACA CCG GGG GGT CGA

TGC AAT CCC CTA GTC CTG GAA TTC ACT GAC 480

GCG GGC AAA AAG GCC AGC TGG GAT GGC CCC

AAA GTA TGG GGA CTA AGA CTG TAC CGA TCC 540

ACA GGG ACC GAC CCG GTG ACC CGG TTC TCT

TTG ACC CGC CAG GTC CTC AAT ATA 594

WHAT IS CLAIMED IS:

1. A retroviral vector particle having a modified envelope polypeptide for targeting the retroviral vector to cells wherein prior to modification the envelope includes a polypeptide selected from the group consisting of (a) a polypeptide having the sequence (SEQ ID NO:1); (b) a polypeptide having the sequence (SEQ ID NO:2); (c) a polypeptide having the sequence (SEQ ID NO: 3); (d) a polypeptide having the sequence (SEQ ID NO: 4); and (e) a polypeptide having the sequence (SEQ ID NO: 5); and wherein, when

(A) prior to modification the envelope includes (SEQ ID NO:1), in the modified envelope at least a portion of (i) amino acids 70 to 92 of (SEQ ID NO: 1); or (ii) amino acids 44 to 114 of (SEQ ID NO: 1); or (iii) amino acids 44 to 131 of (SEQ ID NO: 1); or (iv) amino acids 17 to 182 of (SEQ ID NO:1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells;

(B) and when prior to modification the envelope includes (SEQ ID NO:2), in the modified envelope at least a portion of (i) amino acids 47 to 75 of (SEQ ID NO: 2); or (ii) amino acids 47 to 93 of (SEQ ID NO: 2); or (iii) amino acids 47 to 163 of (SEQ ID NO:2) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells;

(C) and when prior to modification the envelope includes (SEQ ID NO: 3), in the modified envelope at least a portion of (i) amino acids 47 to 75 of (SEQ ID NO: 3); or (ii) amino acids 47 to 93 of (SEQ ID NO: 3); or (iii) amino acids 47 to 163 of (SEQ ID NO: 3) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells;

(D) and when prior to modification the envelope includes (SEQ ID NO: 4) in the modified envelope at least a protion of (i) amino acids 47 to 74 of (SEQ ID NO: 4); or

(ii) amino acids 47 to 92 of (SEQ ID NO: 4); or (iii) amino acids 47 to 154 of (SEQ ID NO: 4) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells;

(E) and when prior to modification the envelope includes (SEQ ID NO: 5), in the modified envelope at least a portion of (i) amino acids 47 to 70 of (SEQ ID NO: 5); or (ii) amino acids 47 to 88 of (SEQ ID NO: 5); or (iii) amino acids 47 to 151 of (SEQ ID NO: 5) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

2. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and at least a portion of amino acids 70 to 92 of (SEQ ID NO: 1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

3. The retroviral vector particle of Claim 2 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and at least a portion of amino acids 74 to 91 of (SEQ ID NO:1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

4. The retroviral vector particle of Claim 3 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and amino acids 80 to 88 of (SEQ ID NO:1) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

5. The retroviral vector particle of Claim 3 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and amino acids 82 to 84 of (SEQ ID NO:1) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

6. The retroviral vector particle of Claim 3 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and amino acids 74 to 80 of (SEQ ID NO:1) are replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

7. The retroviral particle of Claim 3 wherein said targeting polypeptide is alpha-melanotropin stimulating hormone.

8. The retroviral particle of Claim 4 wherein said targeting polypeptide is alpha-melanotropin stimulating hormone.

9. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and at least a portion of amino acids 44 to 114 of (SEQ ID NO: 1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

10. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:1), and at least a portion of amino acids 44 to 131 of (SEQ ID NO: 1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

11. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 1), and at least a portion of amino acids 17 to 182 of (SEQ ID NO: 1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

12. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:2), and at least a portion of amino acids 47 to 75 of (SEQ ID NO: 2) is

replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

13. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:2), and at least a portion of amino acids 47 to 93 of (SEQ ID NO: 2) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

14. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 2), and at least a portion of amino acids 47 to 163 of (SEQ ID NO: 2) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

15. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 3), and at least a portion of amino acids 47 to 75 of (SEQ ID NO: 3) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

16. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 3), and at least a portion of amino acids 47 to 93 of (SEQ ID NO: 3) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

17. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 3), and at least a portion of amino acids 47 to 163 of (SEQ ID NO: 3) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

18. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 4), and at least

a portion of amino acids 47 to 74 of (SEQ ID NO: 4) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

19. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:4), and at least a portion of amino acids 47 to 92 of (SEQ ID NO:4) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

20. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO: 4), and at least a portion of amino acids 47 to 154 of (SEQ ID NO: 4) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

21. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:5), and at least a portion of amino acids 47 to 70 of (SEQ ID NO:5) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

22. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:5), and at least a portion of amino acids 47 to 88 of (SEQ ID NO:5) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

23. The retroviral vector particle of Claim 1 wherein prior to modification the envelope includes a polypeptide having the sequence (SEQ ID NO:5), and at least a portion of amino acids 47 to 151 of (SEQ ID NO:5) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

24. The retroviral vector particle of Claim 1 wherein the targeting polypeptide is a single chain antibody.

25. The retroviral vector particle of Claim 1 wherein prior to modification said retroviral vector particle includes a polynucleotide encoding a heterologous polypeptide which is to be expressed in a targeted cell.

26. A modified polynucleotide encoding a modified retroviral envelope polypeptide for targeting a retroviral vector particle to cells wherein prior to modification the envelope includes a polypeptide selected from the group consisting of (a) a polypeptide having the sequence (SEQ ID NO:1); (b) a polypeptide having the sequence (SEQ ID NO:2); (c) a polypeptide having the sequence (SEQ ID NO:3); (d) a polypeptide having the sequence (SEQ ID NO:4); and (e) a polypeptide having the sequence (SEQ ID NO:5); and

(A) wherein prior to modification, when the envelope includes (SEQ ID NO:1), in the modified polynucleotide at least a portion of (i) the polynucleotide encoding amino acids 70 to 92 of (SEQ ID NO:1); or (ii) the polynucleotide encoding amino acids 44 to 114 of (SEQ ID NO:1); or (iii) the polynucleotide encoding amino acids 44 to 131 of (SEQ ID NO:1); or (iv) the polynucleotide encoding amino acids 17 to 182 of (SEQ ID NO:1) is replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells; and

(B) when the envelope includes (SEQ ID NO:2), in the modified polynucleotide at least a portion of (i) the polynucleotide encoding amino acids 47 to 75 of (SEQ ID NO: 2); or (ii) the polynucleotide enconding amino acids 47 to 93 of (SEQ ID NO: 2); or (iii) the polynucleotide encoding amino acids 47 to 163 of (SEQ ID NO:2) is replaced with a polynucleotide encoding a polypeptide which binds to a ligand or receptor on the targeted cells; and

(C) when prior to modification the envelope includes (SEQ ID NO:3), in the modified polynucleotide at least a portion of (i) the polynucleotide encoding amino acids 47 to 75 of (SEQ ID NO:3); or (ii) the polynucleotide

encoding amino acids 47 to 93 of (SEQ ID NO:3); or (iii) the polynucleotide encoding amino acids 47 to 163 of (SEQ ID NO:3) is replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells; and

(D) when prior to modification the envelope includes (SEQ ID NO:4), in the modified envelope at least a portion of (i) the polynucleotide encoding amino acids 47 to 74 of (SEQ ID NO:4); or (ii) the polynucleotide encoding amino acids 47 to 92 of (SEQ ID NO:4); or (iii) the polynucleotide encoding amino acids 47 to 154 of (SEQ ID NO:4) is replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells; and

(E) when prior to modification the envelope includes (SEQ ID NO:5), in the modified envelope at least a portion of (i) the polynucleotide encoding amino acids 47 to 70 of (SEQ ID NO:5); or (ii) the polynucleotide encoding amino acids 47 to 88 of (SEQ ID NO:5); or (iii) the polynucleotide encoding amino acids 47 to 151 of (SEQ ID NO:5) is replaced with a polynucleotide encoding a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

27. A producer cell for producing a retroviral vector particle having a modified envelope polypeptide, said producer cell including the modified polynucleotide of Claim 26.

28. .A method of effecting a gene therapy treatment in a host, comprising:

administering to a host the retroviral vector particles of Claim 25 in an amount effective to produce a therapeutic effect in said host.

29. A modified envelope polypeptide wherein prior to modification the envelope includes a polypeptide selected from the group consisting of (a) a polypeptide having the

sequence (SEQ ID NO:1); (b) a polypeptide having the sequence (SEQ ID NO:2); (c) a polypeptide having the sequence (SEQ ID NO: 3); (d) a polypeptide having the sequence (SEQ ID NO:4); and (e) a polypeptide having the sequence (SEQ ID NO.5); and wherein,

(A) when prior to modification the envelope includes (SEQ ID NO:1), in the modified envelope at least a portion of (i) amino acids 70 to 92 of (SEQ ID NO: 1); or (ii) amino acids 44 to 114 of (SEQ ID NO: 1); or (iii) amino acids 44 to 131 of (SEQ ID NO: 1); or (iv) amino acids 17 to 182 of (SEQ ID NO:1) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells; and

(B) when prior to modification the envelope includes (SEQ ID NO:2), in the modified envelope at least a portion of (i) amino acids 47 to 75 of (SEQ ID NO: 2); or (ii) amino acids 47 to 93 of (SEQ ID NO: 2); or (iii) amino acids 47 to 163 of (SEQ ID NO:2) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells and

(C) when prior to modification the envelope includes (SEQ ID NO:3), in the modified envelope at least a portion of (i) amino acids 47 to 75 of (SEQ ID NO: 3); or (ii) amino acids 47 to 93 of (SEQ ID NO:3); or (iii) amino acids 47 to 163 of (SEQ ID NO:3) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells; and

(D) when prior to modification the envelope includes (SEQ ID NO:4), in the modified envelope at least a portion of (i) amino acids 47 to 74 of (SEQ ID NO:4); or (ii) amino acids 47 to 92 of (SEQ ID NO:4); or (iii) amino acids 47 to 154 of (SEQ ID NO:4) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells; and

(E) when prior to modification the envelope includes (SEQ ID NO:5), in the modified envelope at least a portion of (i) amino acids 47 to 70 of (SEQ ID NO:5); or (ii) amino acids 47 to 88 of (SEQ ID NO:5); or (iii) amino acids 47 to 151 of (SEQ ID NO:5) is replaced with a targeting polypeptide which binds to a ligand or receptor on the targeted cells.

30. A retroviral plasmid vector including the polynucleotide of Claim 26.

31. A method of generating retroviral vector particles, comprising:

(a) transfecting a cell line selected from the group consisting of (i) a pre-packaging cell line including polynucleotides encoding the gag and pol retroviral proteins; and (ii) a packaging cell line including polynucleotides encoding the gag, pol, and env retroviral proteins with the retroviral plasmid vector of Claim 30 to form a producer cell line; and

(b) culturing said producer cell line to generate retroviral vector particles.

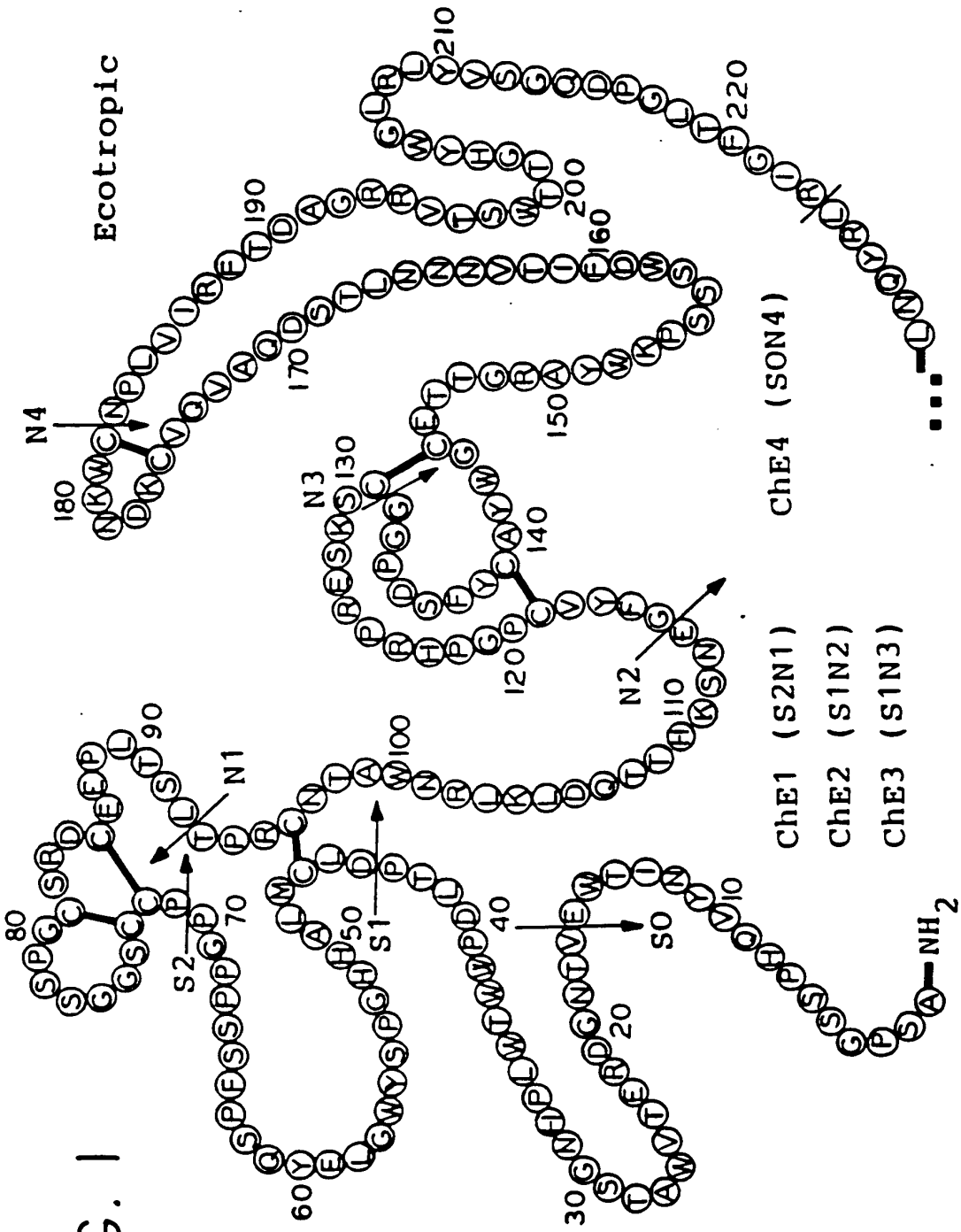

Ecotropic
ChE1 (S2N1)
ChE2 (S1N2)
ChE3 (S1N3)
ChE4 (SON4)
N1
N2
N3
N4
S0
S1
S2
NH2

FIG. 1

FIG. 2

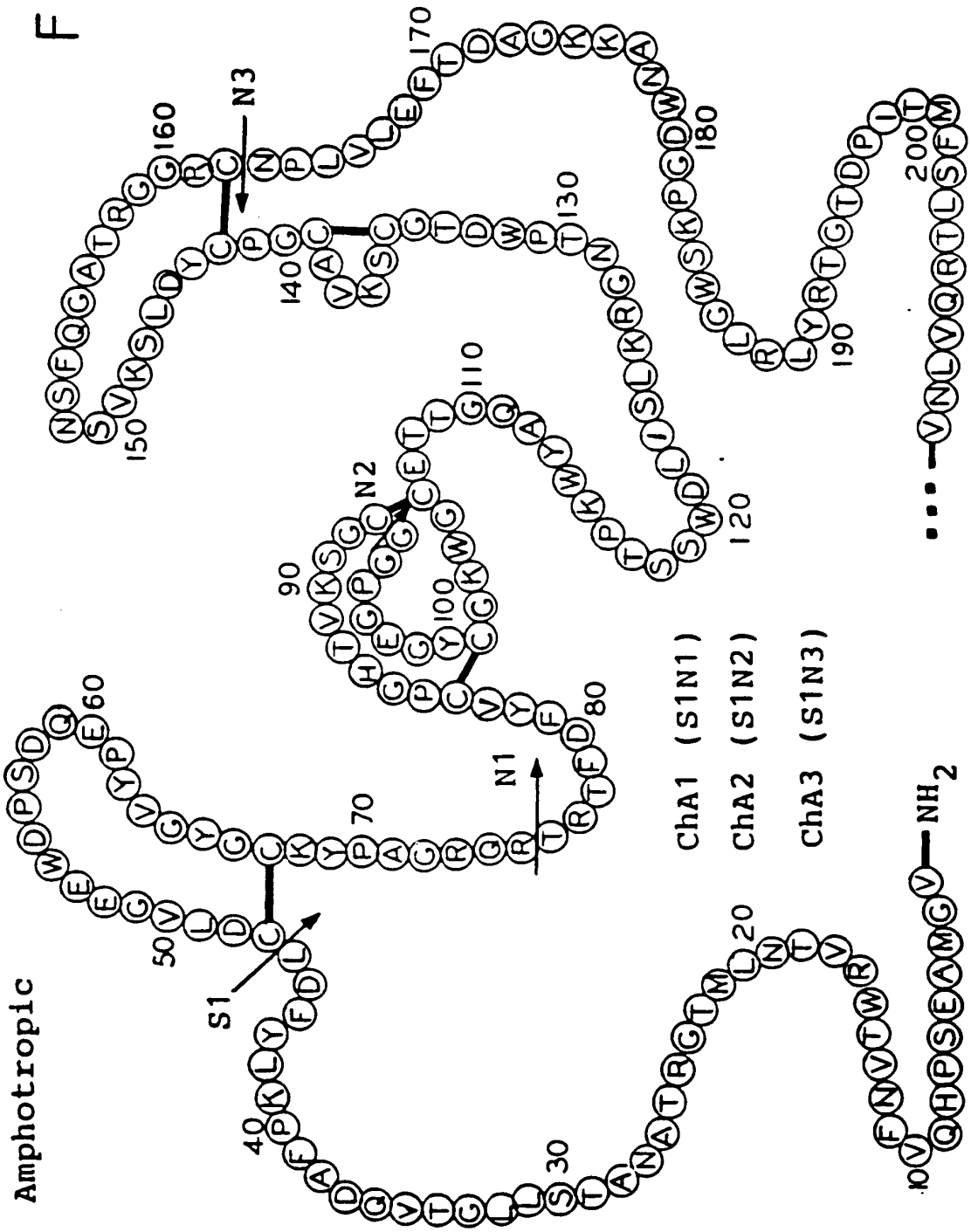
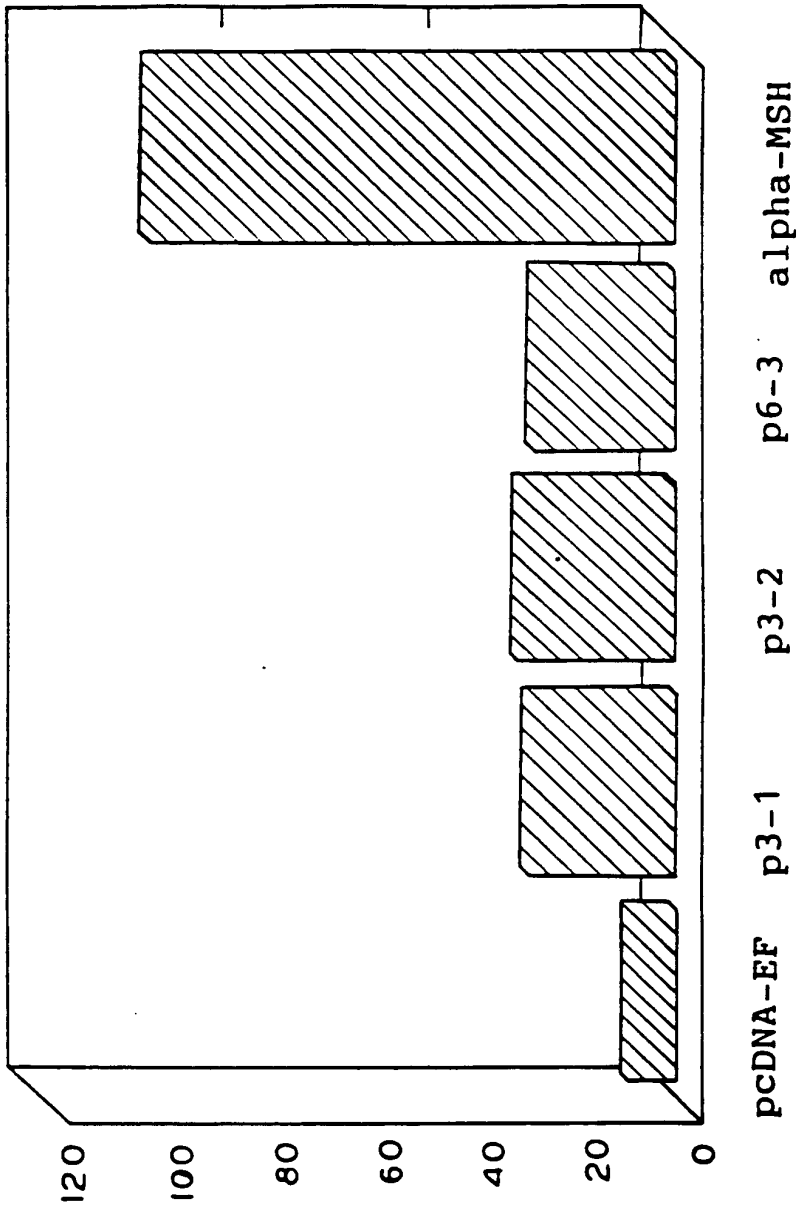


FIG. 3


pCDNA-EF
p3-1
p3-2
p6-3
alpha-MSH
0
20
40
60
80
100
120
Relative percentage melanin produced

INTERNATIONAL SEARCH REPORT

International application No
PCT/US96/03908

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C12N 15/00; A61K 48/00
US CL :435/320.1; 514/44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/320.1; 514/44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, MEDLINE, EMBASE, BIOSIS, CAPLUS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y	ETIENNE-JULAN et al. Cell targeting by murine recombinant retroviruses. Bone Marrow Transplantation. 1992, Vol. 9, Suppl. 1, pages 139-142, see entire document.	1-31
Y	MILLER et al. Targeted vectors for gene therapy. FASEB J. February 1995, Vol. 9, pages 190-199, see entire document.	1-31
Y	KASAHARA et al. Tissue-Specific Targeting of Retroviral Vectors Through Ligand-Receptor Interactions. Science. 25 November 1994, Vol. 266, pages 1373-1376, see entire document.	1-31

[X] Further documents are listed in the continuation of Box C [ ] See patent family annex.

*	Special categories of cited documents	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 06 JUNE 1996	Date of mailing of the international search report 13 JUN 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No (703) 305-3230	Authorized officer D. CURTIS HOGUE, JR. Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No
PCT/US96/03908

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MORGAN et al. Analysis of the Functional and Host Range-Determining Regions of the Murine Ecotropic and Amphotropic Retrovirus Envelope Proteins. J. Virology. August 1993, Vol. 67, No. 8, pages 4712-4721, see entire document.	1-31